EFFECT OF COLLECTION METHOD AND ARCHIVING CONDITIONS ON THE SURVIVABILITY OF VEGETATIVE AND SPORE FORMING BACTERIA

A Thesis

by

ASMAA SADEK KASSAB

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2009

Major Subject: Mechanical Engineering

EFFECT OF COLLECTION METHOD AND ARCHIVING CONDITIONS ON THE SURVIVABILITY OF VEGETATIVE AND SPORE FORMING BACTERIA

A Thesis

by

ASMAA SADEK KASSAB

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Bing Guo
Committee Members,	Andrew R. McFarland
	Maria D. King
	Yassin A. Hassan
Head of Department,	Dennis L. O'Neal

August 2009

Major Subject: Mechanical Engineering

ABSTRACT

Effect of Collection Method and Archiving Conditions on the Survivability of Vegetative and Spore Forming Bacteria. (August 2009)

Asmaa Sadek Kassab, B. Sc., Alexandria University, Egypt

Chair of Advisory Committee: Dr. Bing Guo

To ensure effective detection of bio-particles, it is crucial to understand the effects of collection method and archiving conditions on the survivability of bio-aerosols, consequently, the survivability of the spore-forming *Bacillus globigii* (BG) and MG1655 *Escherichia coli* (*E. coli*), was determined after collection. The survivability was defined as the culturable fraction of the archived bacteria/culturable fraction of the as-collected bacteria. The bacteria were aerosolized for up to four days at room temperature (RT, 25˚C) and at 4˚C and collected in a 100 L/min wetted wall cyclone (WWC) and a 12.5 L/min SKC BioSampler. Aqueous solutions of 0.01% Tween-20 and 30% Ethylene Glycol (EG), with or without 0.5% ovalbumin (OA), were used as the collection fluids. Antifoam B (A-F), at a concentration of 0.2% (V:V) was added to the BG samples containing OA.

In general, samples archived at 4˚C showed higher survivability than at RT. The survivability were more stable in EG than in Tween-20 especially for BG, very likely

due to the surfactant effect of the Tween-20, which would remove the spore coat and initiate germination.

In the WWC, adding OA significantly increased the survivability of BG in EG and in Tween-20, especially at RT. Similar effect of OA was found for *E. coli* samples stored in EG, suggesting that OA might be beneficial in maintaining the survivability. Adding A-F increased the survivability of BG in EG. In the SKC, neither the addition of OA nor A-F seems to have a beneficial effect on the survivability of the spores in EG samples.

The best collection fluid for maintaining survivability in the WWC is EG+A-F for BG, and EG+OA for *E. coli*. However, in the SKC, EG is the best for BG collection and Tween-20 for *E. coli*.

Viability transfer ratios, VTR, (cells surviving collection at time zero/viable cells aerosolized) were calculated for both devices. A performance ratio was calculated as the VTR of the WWC/VTR of the SKC. The geometric mean of the performance ratio is 1.51±0.83 for BG and 2.60±0.16 for *E. coli*, indicating that viability transfer ratio of the WWC is typically higher than that of the SKC.

DEDICATION

To my recently born son, Ramy, and my beloved husband, Ahmed

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Professor Bing Guo, and my committee members, Professors A. McFarland, Y. Hassan and Dr. M. King, for their continuous guidance and support throughout my research.

A special thanks to Dr. M. King for her understanding, help and love that she is always ready to provide in our Aerosol Technology Laboratory that kept me going even in the hard times.

Finally, I would also like to thank my beloved parents, Sadek and Hanaa, my lovely sisters, Heba and Aya, and my parents-in-law, Noor and Sohair for their love, encouragement, and for standing by me at the hardest of times.

NOMENCLATURE

AD	Aerodynamic Diameter
A-F	Antifoam
AGI	All Glass Impinger
aka	Also known as
ANOVA	Analysis of Variance
a_r	Rate of Atomization
ARL	Applied Research Laboratory
ATL	Aerosol Technology Laboratory
BG	*Bacillus globigii*
CFU	Colony Forming Unit
CFU_0	Colony Forming Unit as Collected
CFU_{avg}	Average Colony Forming Unit
CFU_i	Colony Forming Unit for an Archived Day
CFU_t	Total Colony Forming Unit
$C_{m,air}$	Relative Mass Concentration
C_s	Concentration of the Stock
DF	Degree of Freedom
Dil	Sample Dilution
EG	Ethylene Glycol
F_c	Correction Factor

g	Number of Groups
LB	Luria Bertani
LPM	Liter per Minute (L/min)
L_r	Liquid Loss Rate
MS	Mean Square
$MS_{Residual}$	Mean Square Residual
MSR	Mean Square Residual Ratio
MQ	Millique Water
N	Number of Observations or Sample Size
n	Number of Replications
OA	Ovalbumin
OD	Optical Density
PBST	Phosphate Buffer Stain Triton
PR	Performance Ratio
Q	Air Flow Rate
Q_s	Sampling Flow Rate
RT	Room Temperature (25˚C)
r	Number of Rows
S	Survivability
SS_c	Sum of Squares along Columns
SS_{cg}	Sum of Squares along Columns and Groups
SS_{cr}	Sum of Squares along Columns and Rows

SS_{crg}	Sum of Squares along Columns, Rows and Groups
SS_g	Sum of Squares along Groups
SS_r	Sum of Squares along Rows
SS_{residual}	Residual Sum of Squares
SS_{rg}	Sum of Squares along Rows and Groups
SS_{total}	Total of Sum of Squares
STDV	Standard Deviation
T	Summation of all Sample Responses
t	Sampling Period
T_r	Summation of Sample Responses by Row
T_c	Summation of Sample Responses by Column
T_g	Summation of Sample Responses by Group
T_{rg}	Summation of Sample Responses by Row and Column
T_{cg}	Summation of Sample Responses by Column and Group
T_{cr}	Summation of Sample Responses by Column and Row
T_{crg}	Summation of Sample Responses by Column, Row and Group
TSA	Tryptic Soy Agar
TW-20	Tween-20
V	Solution Volume
V_c	Collected Volume
V_p	Plated Volume
VTR_{SKC}	Viability Transfer Ratio for SKC

VTR_{WWC}	Viability Transfer Ratio for WWC
WWC	Wetted Wall Cyclone
x	Value of Sample
$\eta_{A\text{-}H}$	Aerosol-to-Hydrosol Collection Efficiency

TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Page

Page

CHAPTER I

INTRODUCTION

1.1 General Background

An aerosol is a suspension of fine solid particles or liquid droplets in a gas, with particle sizes from less than one micrometer to one hundred micrometers, including bio-aerosols that are aerosols of biological origin. Typically the particle size range of interest in bio-aerosol sampling is 1 to 10 μm AD (Aerodynamic Diameter). They include viruses and living organisms, such as bacteria and fungi (Hinds, 1999). The presence of naturally occurring bioaerosol particles is usually the result of dispersal from a site of colonization or growth. Currently, bio-aerosol characterization has become an important issue because of their related health effects. The health effects of bio-aerosols including infectious diseases, acute toxic effects, allergies and cancer coupled with the threat of bioterrorism and SARS (Severe Acute Respiratory Syndrome) have led to increased awareness on the importance of bio-aerosols (Srikanth *et al*., 2008; Jensen and Schafer, 1998).

Aerosols play a large role in a multiplicity of different production processes. Airborne particles, especially pathogenic microorganisms and other biological materials, are potential public and industrial health hazards. To ensure the effective protection of

---

This thesis followed the style and format of *Aerosol Science and Technology*.

both general and occupational populations from the adverse effects of airborne hazards, reliable methods for air sampling are required. In general, field evaluations in the natural environment are likely to provide very limited information with uncontrolled or unknown natural aerosol concentrations, particle sizes, and flora (Henningson and Ahlberg, 1994). Thus, it is necessary to use a test aerosol in chamber to assess bio-aerosol sampler performance (Griffiths and DeCosemo, 1994).

Bio-aerosols can be divided into two main groups: viable and nonviable. Viable microorganisms are metabolically active (living) organisms with the potential to reproduce. Viable microorganisms may be divided into two subgroups: culturable and nonculturable. Culturable organisms reproduce under controlled conditions. Nonculturable organisms do not reproduce in the laboratory because of intracellular stress or because the conditions (e.g., culture medium or incubation temperature) are not conducive to growth. Viable bio-aerosol sampling involves collecting a bio-aerosol; however, typically only culturable microorganisms are enumerated and identified, thus leading to an underestimation of bio-aerosol concentration. Nonviable microorganisms are not living organisms; as such, they are not capable of reproduction. When sampling for culturable bacteria and fungi, the bio-aerosol is generally collected by impaction onto the surface of a broad spectrum solid medium (agar), filtration through a membrane filter, or impingement into an isotonic liquid medium (water-based). Organisms collected by impaction onto an agar surface may be incubated for a short time, replica-plated (transferred) onto selective or differential media, and incubated at different temperatures for identification and enumeration of microorganisms (Tortora *et al.,*

1989). The most common bio-aerosols are bacterial and fungal spores. Bacteria are single-celled organisms with size from 0.3 to 10 μm. In the airborne state, the microorganisms however, are not very frequently found as single cells. More often they will be found in aggregates (clustered to each other) or attached to non-viable particles (Akers and Won, 1969). They are mostly water, with a density that ranges from 1000 to 1500 kg/m^3, and they are spherical or rod shaped. Some bacteria release spores called endospores. These are hardy, dormant version of the bacteria, 0.5 to 3 μm in size (Hinds, 1999). Endospores are very resistant to various environmental stresses Sneath (1986).

Samplers for microbiological aerosols have to be evaluated in the same way as other aerosol samplers, but an additional vital factor to determine here is the ability of the samplers to preserve the viability of the airborne microorganisms (Henningson and Ahlberg, 1994), which is usually measured by culturability. The culturability is defined as the count of culturable bacteria particles in a collected sample. It is a measure of how well the microorganisms survive the sampling process which can be determined by cultivation, in this study named as colony forming unit (CFU). The overall sampling efficiency of aerosol samplers with different designs may differ significantly from each other because of the different physical collection efficiency and the stress imparted to the microorganisms. Bio-aerosol resistibility, sampling time, and flow rate are the most important factors to affect microbial collection and survival in bio-aerosol samplers (Macher and Willeke, 1993).

The aerosol is collected by separating the particles from the airstream by utilizing different physical forces. These forces also constitute a base for classification of

samplers (Hinds, 1999; Lehtimaki and Willeke, 1993) in inertial devices (impactors, cyclones, and centrifuges), filters and other types of samplers. In cyclones the aerosol is forced into a centrifugal motion and the particles in the airstream with high enough inertia are deposited on the wall of the sampler (Henningson and Ahlberg, 1994). In that environment, Wathes and Randall (1988) found that the cyclone appeared to be the ideal sampler. One commonly used method for measuring airborne microorganisms is liquid impingement. In this method, the airborne microorganisms are drawn through a nozzle and impinged vertically into a liquid reservoir. Liquid as the collection medium in an impinger has attractive features relative to the nutrient agar medium in an impactor (Lin *et al.*, 2000).

For optimal recovery, the collection fluid used in the bio-aerosol samplers could glycerol or ethylene glycol and surfactants such as Tween-20. The polyethylene glycols are good solubilizers and possibly they solubilize certain of the involved spore components. On the other hand, it has been suggested for vegetative cells, that the polyethylene glycol causes a detrimental change in their culture (Robison and Weinswig, 1968). Hamoen and Errington (2003) discovered that the addition of the detergent Tween-20 to the growth medium substantially stimulated germination and countered adverse effects of SDS (sodium dodecyl sulfate) extraction. Additionally, an inhibitory effect of Tween in elevated concentration (>0.01%) was found by Sun *et al.* (2008) that causes cell membrane damage in Bacillus spores. Ovalbumin may provide a protective coating on the bacteria surface and also a source of nutrients. According to Tobian *et al*.

(2004), *E. coli* is capable of binding ovalbumin mostly via heat shock proteins that are increasingly expressed by the bacteria in stress.

During the sampling process of bio-aerosols in different collection devices the bacteria have to maintain their integrity to be analyzed in a detector which may involve an archiving period extending to five days at different temperatures. The extent of mechanical and osmotic stress that the particles have to endure in an air-to-hydrosol collector is influenced by several factors, such as the airflow, the length of sampling and the type of collection fluid.

1.2 Objective of the Present Study

The objective of this study is to determine the effects of collection methods and archiving conditions on the survivability of biological aerosol particles using a 100 LPM wetted wall cyclone (McFarland, 2009) and an aerosol-to-hydrosol impinger, the 12.5 LPM SKC BioSampler (SKC Inc., Eighty Four, PA).

Two types of bacteria were used as the viable particles, the spore forming *Bacillus subtilis var. niger* (aka *Bacillus globigii*, (BG), aka *Bacillus atrophaeus*), classified as a gram positive bacteria and the non-sporulating, vegetative MG1655 *Escherichia coli*, classified as a gram negative bacteria. The vegetative *E. coli* is more sensitive to environmental stresses than the BG spores (Lin *et al.,* 2000; Sneath, 1986).

To study the effect of collection fluid composition on the survivability, 0.01% Tween-20 and 30% Ethylene Glycol (EG), with or without the addition of 0.5% chicken egg ovalbumin, OA (Sigma, St. Louis, MI), were used as the collection fluids.

Additional tests were also conducted to study the effect of adding 0.2% of Antifoam B Silicone Emulsion (Sigma, St. Louis, MO) to BG samples (Daly *et al*., 2005)

In addition, several types of factors were taken into considerations in this study, such as the maintenance of survivability of as-collected samples. An archiving study was conducted for a four day period following the day of collection (five days in total) taking into consideration the effect of two different temperatures, the room temperature (RT, 25˚C) and the 4˚C.

To indicate the stress associated with the sampling process, a viability transfer ratio, VTR, was determined for both the WWC and the SKC, where the VTR is the ratio of the number of viable bacteria that survive the collection process to the number of viable bacteria that are aerosolized. First the aerosol to hydrosol collection efficiency of the WWC and the SKC BioSampler was determined using nonviable fluorescently tagged Polystyrene Latex Microspheres (PSL) for 1μm AD particle size using the 6-jet Collison nebulizer at 20 psig. Second, the liquid loss rate measured experimentally. In a work published in 1977, the aerosol output and the liquid consumption of a Collison nebulizer were measured. The results suggested that about 58% of the liquid loss was due to atomization, the rest being evaporation (Young *et al.* 1977). The aerosol output of a jet nebulizer Mefar^{TM} has been measured in a number of works. The actual aerosol output (measured based on solute loss) was from 50% - 76% (Dennis *et al.* 1992), 54% - 73% (Ward *et al.* 1998), 44% - 72% (Gatnash *et al.* 1998). In 1999, a review suggested that in general the aerosol output would be approximately 50% of that determined by liquid weight loss (Ward *et al.* 1999).

CHAPTER II

DIFFERENT TYPES OF COLLECTORS

2.1 100 LPM Wetted Wall Cyclone (WWC)

2.1.1 Development of the Wetted Wall Cyclone

In 1933, the Wells centrifuge with a solid collection surface was constructed (Wells, 1933); however, not until 1969 was a cyclone sampler designed where microorganisms were collected in a liquid (Errington *et al.*, 1969). This cyclone separator was operated at a high volumetric flow rate and concentrated the particulate into a small liquid effluent flow rate. White *et al.* (1975) developed an axial flow cyclone for concentrating bio-aerosol particles from a flow rate of 950 LPM of aerosol to a continuous liquid flow rate between 1 and 2 mL/min. Later a modified cyclone was tested for collection stage efficiency with latex particles by Henningson *et al.,* 1988. The White cyclone was further developed by Black and Shaw (2002) who opted for an air flow rate of 900 LPM and a liquid effluent flow rate of 1.6 mL/min.

At the Aerosol Technology Laboratory (ATL) at Texas A&M University, experimental and numerical investigations were undertaken related to developing and improving the performance of the Black and Shaw type wetted wall cyclone (Moncla, 2004). The cyclone was shown to exhibit entrainment of liquid from the internal wall into the exhaust airflow stream (liquid bypass). Ostensibly, this was due to a ring of

recirculation liquid was observed that spins near the skimmer tip during cyclone operation.

In a further investigation Phull (2005) suggested alternate methodologies for liquid injection and showed that the aerosol-to-hydrosol (A-H) collection efficiency with the air-blast atomizer was higher than that of any other methodology he tested because the air-blast atomizer wets the impacting wall evenly, so that all particles that strike the wall are able to be collected. Therefore, it was concluded that the air-blast atomization would be the most effective way (same approach as used in this study).

2.1.2 Principle of Particle Collection Using Wetted Wall Cyclone

A wetted wall cyclone shown in Figure A.1 is a device that delivers hydrosol in a single stage from which real-time detection of airborne particles can be readily achieved. It is a tangential-inlet flow, axial outlet flow bio-aerosol sampler that continuously collects particles onto a flowing liquid film. Moreover, it is a concentration device that transfers particles to the liquid phase to facilitate subsequent analysis. The aerosol particles are impacted onto the input flowing liquid film. The shear force created by the airflow carries the liquid to a skimmer where the hydrosol and air flows are separated, and the hydrosol is extracted from the system.

As shown in Figure A.2, inlet air flow containing the aerosol introduced through the flange at the top is accelerated through its passage through a convergence section and impacts the wall of the cyclone body. The vortex finder helps to stabilize the vortex air flow inside the cyclone. The rotating vortex flow is then extracted from the cyclone by a

blower. An atomizer located approximately midway in the inlet section of the cyclone provides the liquid spray to uniformly wet the impacting wall. As shown in Figure A.3 the atomizer utilizes two needles for uniform dispersion; one for liquid and the other one for air. The angle between the two needles has to be well adjusted to ensure that the impacting wall will be uniformly covered with the liquid spray. Deposits on the cyclone wall recovered by the sprayed liquid are carried downstream and extracted through the sample extraction port. Figure A.4 shows the 100 LPM WWC used in this study.

2.2 SKC BioSampler Impinger

2.2.1 Background

One commonly used method for measuring airborne microorganisms is liquid impingement. The liquid impingers are a special type of impactor. Impingers are useful for the collection of culturable aerosols (White *et al*., 1975; Lembke *et al.,* 1981; Henningson *et al.,* 1988).

In 1964 two samplers were recommended as standards for sampling of microbiological aerosols: the Andersen 6-stage sampler and the all glass impinger-30 (AGI-30) (Brachman *et al.,* 1964). They also have been suggested as the samplers of choice for the collection of viable microorganisms by the International Aerobiology Symposium and the American Conference of Governmental Industrial Hygienists (Jensen *et al.,* 1992; Brachman *et al.,* 1964; Chatigny *et al.,* 1989). AGI-30 with its

simple construction and relative high recovery is the most efficient impinger for monitoring in bio-processing plants according to (Salusbury *et al.,* 1988).

The airborne microorganisms are drawn through a nozzle and impacted vertically into a liquid reservoir. One of the problems with traditional liquid impingement is that the air flow produces bubbling in the liquid reservoir, which may cause previously collected particles to be re-aerosolized (Grinshpun *et al.,* 1997; Lin *et al.,* 1997). Impingers are commonly used with water or liquids having viscosity and surface tension characteristics similar to water. If the collection liquid is primarily water, it may readily evaporate during sampling.

The AGI-30 operates at a nominal flow rate 12.5 LPM (Macher *et al.* 1995). According to Lin, *et al*. (2000) the device now known as the BioSampler (SKC Inc., Eighty Four, PA), Figure A.5, provides equivalent or better microbial recovery for the *B. subtilis* and *P. fluorescens* compared to the AGI-30. Originally, the BioSampler was first labeled "Swirling Aerosol Collector'' (Willeke *et al.,* 1998) and was developed to improve the collection efficiency for microorganisms. The device is also used with other collection fluids than one that is primarily water, as a means of prolonging the sampling period.

2.2.2 Principle of Particle Collection Using SKC All Glass Impinger

The BioSampler is composed of three parts: the inlet section, the nozzle section, and the collection vessel section. As shown in Figure A.6a, the ambient aerosol is sampled horizontally into the inlet. The downward aerosol flow is then split into three

nozzle flows. Each nozzle has a sonic orifice, which allows about 4.2 LPM of ambient air to pass through if the sampling vacuum pump establishes a downstream pressure of 0.5 atm or less. Each of the nozzle orifices is directed at an identical angle toward the curved inner surface. Thus, the aerosol particles are thrown at an angle toward the surface and are removed by oblique impaction. The presence of three angular nozzles establishes swirling air motion in the collection vessel. The swirling air flow entrains the liquid and carries it upward into the region where the aerosol flows from the nozzles reach the inner vessel surface. Thus, the aerosol particles are removed into the liquid film, which carries them down into the liquid reservoir, as shown in Figure A.6b. Drain tips on the nozzles prevent liquid droplets from being drawn into the air jets. The air exits through a single exhaust port.

CHAPTER III

EXPERIMENTAL METHODOLOGY

3.1 Test Microorganisms

In this study two types of bacteria were used as the test aerosol particles: single spores of *Bacillus subtilis* (aka *Bacillus globigii*, BG, and *Bacillus atrophaeus*), (Figure A.7), which are classified as a gram positive bacteria; and, single cells of non-sporulating, mid-log phase fresh vegetative cells of MG1655 *Escherichia coli* (Figure A.8), which are classified as a gram negative bacteria, The vegetative MG1655 *E. coli* is more sensitive to environmental stresses than the BG spores (Lin *et al.*, 2000; Sneath, 1986).

3.1.1 *Bacillus globigii*

Dry *Bacillus globigii* spore powders were acquired from the U.S. Army Edgewood Laboratories (courtesy Dr. Edward W. Stuebing of the Edgewood Research, Development, and Engineering Center; Aberdeen Proving Ground, MD).

To generate single spores of *Bacillus globigii*, 25 mg of the BG lyophilized powder was re-suspended in 5 mL of 5% PBST (Phosphate Buffer Saline with 0.1% Triton X-100, pH 7.4) and centrifuged using Centrifuge 5804 (Eppendorf, Hamburg, Germany) at 2880 g for 9 minutes in order to remove traces of the culture medium. The

supernatant was aspired and the pellet was resuspended in 500 mL of 5% PBST to be used as the stock suspension.

3.1.2 *Escherichia coli*

For MG1655 *Escherichia coli* mid-log phase (OD_{600}=0.5) cultures were grown in Luria Bertani (LB) medium (Sambrook *et al.,* 1989) for about three hours at 37^{o}C and at 150 rpm in the incubator (Imperial III General Purpose Thermo Scientic Lab-Line Incubators). The cells were harvested by pelleting them at 2880 g for nine minutes, diluted in sterile milliQ water (MQ water), containing 5% PBST to be used as the stock suspension.

3.2 Experimental Setup

Figure A.9 displays the test setup used to evaluate the particle collection characteristics of the different types of collectors.

1. A 100 LPM Wetted Wall Cyclone (WWC) unit, developed by the Aerosol Technology Laboratory (ATL) and the Applied Research Laboratory (ARL), UT Austin, and fabricated by TSI Inc., (Shoreview, MN) that continuously collects particles onto a flowing liquid film.
2. 225-9594 SKC Biosampler (SKC Inc., Eighty Four, PA), in which the airflow passes through three tangential nozzles and collects the particles in a batch of liquid.

The collection tests were performed in the Biological Safety Cabinet BSL 2 (Model NU-425, NUAIRE, Plymouth, MN) under constant airflow and at room temperature (RT) using the setup shown in Figure A.9, with three sampling sequences per collection fluid (12 samples in total) to determine any changes in the survivability of the bacteria.

3.2.1 100 LPM Wetted Wall Cyclone (WWC)

Before staring the experiment, a three step washing procedure was applied to the 100 LPM WWC to prevent contamination in the collected samples, Two-minute washing with 2% bleach in MQ water to kill bacterial contamination from previous experiments followed by two-minute washing with isopropanol to eliminate the bleach effect and a final 30 minute washing with 0.01% Tween-20 to ensure that the cyclone was clean and ready for the experiment.

The bacteria were sampled by the 100 LPM WWC for ten minute periods, and were generated using a six-jet Collison nebulizer (Model CN60, BGI Incorporated, Waltham, MA) with a fresh batch (30 mL) of the stock suspension used for each test. Additional two minute collections after the nebulizer was turned off were also performed to recover hydrosol particles remaining in the cyclone and liquid flow lines. The Collison nebulizer works by using compressed air, which passes through a venturi, where liquid is aspirated into a sonic velocity air jet and atomized into droplets. This

liquid/air jet is impacted against the inside wall of the jar to remove the larger droplets. The air pressure for the nebulizer was set at 138 kPa (20 psig).

The 100 LPM WWC was run using PumpLink software (Cavro Scientific Instruments Inc., Sunnyvale, CA) at the inflow rate of 250 µl/min and effluent rate of 160µl/min with different collection liquid solutions, 0.01% Tween-20 and 30% Ethylene Glycol (EG) with or without 0.5% OA, chicken egg ovalbumin (Sigma, St. Louis, MI). The ovalbumin content of the hydrosols was analyzed by the NanoOrange fluorescent protein kit (Molecular Probes, Portland, OR) using a Quantech-Turner fluorometer at excitation/emission NB 490/NB 590. Antifoam B Silicone Emulsion (Sigma, St. Louis, MO) was added to the BG WWC samples at a concentration of 0.2% (V:V). The liquid, at a predetermined inflow rate, was provided to the cyclone by a CAVRO pump (Model XP 3000, Cavro Scientific Instruments Inc., San Jose, CA). A micro-diaphragm liquid pump (Model PML 5239-NF31, KNF Neuberger, Trenton, NJ) was used to extract the hydrosol from the 100 LPM cyclone. Another micro-diaphragm pump served as a compressed air source for the air-blast atomizer. A blower (Model 119104, Ametek, Inc. Paoli, PA) provided the air flow through the system. Between samples, a three minute washing period was inserted where the cyclone was run in a sampling cycle with the nebulizer turned off, and increased to ten minutes washing when switching between two different solutions.

3.2.2 SKC BioSampler

The two types of bacteria were also collected with SKC BioSamplers, which contained 15 mL of the same liquids used in the WWC tests. A six-jet Collison nebulizer was used to disseminate the particles with a fresh batch (30 mL) of the stock suspension for each sample collection. Ten minute aerosolization periods were used with the SKC operated at the standard sampling flow rate of 12.5 LPM. A water wash was applied when changing collection fluids.

3.2.3 Collection Fluids

The hydrosol samples were weighed (AB104-S Analytical Balance, Mettler-Toledo, Inc., 1900 Polaris Parkway, Columbus, OH, 43240). To study the effect of temperature on the survivability of bacteria, each hydrosol sample was divided into two equal volumes, which were stored at room temperature (RT, 25˚C) and 4^{o}C. The culturable counts of the liquid samples drawn from the storage suspensions were determined by plating 100 μL volumes at appropriate dilutions on Difco Tryptic Soy Agar (TSA) plates (Becton Dickinson Co., Sparks, MD), (Jones, 1979; Buck, 1979, Chatigny *et al.,* 1989) at 24-hour intervals over a five-day period, incubating them overnight at 37˚C, and counting the culturable particles as Colony Forming Units (CFU) using the colony counter (Bantex, Burlingame, CA).

3.2.4 Aerosol to Hydrosol Collection Efficiency of the Sampling Devices

To prepare the Master Suspension, 60 mL batch of PSL (Duke Scientific, Palo Alto, CA and Bangs Lab, Fishers, IN) was added to 540 mL of distilled water. To have consistent PSL concentration output from the nebulizer, fresh aliquots of 30 ml PSL Master Suspension were used for each ten min aerosolization and collection period. At the end of the test, the leftover suspension was placed in a recycled PSL suspensions container to be reused as the master suspension for other sets of tests later.

The samples that were collected in the receiver tube were transferred to a glass container. The liquid was vaporized with a heat gun (Type 3458, STEINEL, Bloomington, MN) and then a 10 ml of ethyl acetate was added to the container to dissolve the dried PSL. This solution was set aside for about half an hour to allow the dissolution process to reach completion.

For a reference filter, a 47 mm glass fiber filter, GF, Type A/E (Pall, East Hills, NY) was used to collect the PSL particles, following the same procedures as in the collectors .Then the filter was transferred into a container that contain 10 ml of ethyl acetate to dissolve the PSL from the filter and a threaded lid for the container had to be on during soaking. Then the container was left for approximately half an hour to ensure proper mixing. A fluorometer (Model FM109515, Quantech, Barnstead International, Dubuque, IA) was used to quantify the fluorescence of the collectors effluent samples.

CHAPTER IV

ANALYSIS

Samplers for microbiological aerosols have to be evaluated in the same way as other aerosol samplers, but an additional vital factor to determine is the capacity of the samplers to preserve the viability of the airborne microorganisms.

4.1 Survivability Calculations

4.1.1 Survivability

The survivability was defined as the culturability in the archived sample divided by the culturability in the as-collected sample.

$$S = \frac{CFU_i}{CFU_0} \quad (4.1)$$

where

S is the survivability.

CFU_i is the archived colony forming unit for a specified collection fluid and archived day.

CFU_0 is the total colony forming unit for a specified collection fluid as collected (at time zero). Its value is 100% for as-collected samples, day one.

4.1.2 Culturability

Culturability is the count of culturable bacteria particles in a collected sample an is a measure of how well the microorganisms survive the sampling process which can be determined by cultivation, in this study named as colony forming unit (CFU), and can be calculated based on the following equations:

$$CFU_t = \frac{CFU_{avg} * V_c * Dil}{V_p} \quad (4.2)$$

where

CFU_t is the total colony forming unit

CFU_{avg} is the average colony forming unit

V_c is the collected volume (ml)

Dil is the dilution

V_p is the plated volume (ml)

4.2. Viability Transfer Ratio Calculations

4.2.1 Viability Transfer Ratio and Performance Ratio

The viability transfer ratio, VTR is defined as:

$$VTR = \frac{CFU_0}{\eta_{A-H} C_s Q_s t} \quad (4.3)$$

where

CFU_0 is total CFU in the sampler collection liquid at time zero

$\eta_{A\text{-}H}$ is the aerosol-to-hydrosol collection efficiency for 1 μm particle size

Q_s is the sampling flow rate (l/min)

C_s is the concentration of viable organisms in the stock (CFU/l)

t is the sampling period when the aerosol was collected (min)

A performance ratio, PR, can be used to compare the degradation of viability suffered by organisms in two samplers, e.g., the WWC and the SKC. This ratio is:

$$PR = \frac{VTR_{WWC}}{VTR_{SKC}} \quad (4.4)$$

where

VTR_{WWC} is the viability transfer ratio of the WWC

VTR_{SKC} is the viability transfer ratio of the SKC

Assuming the values of viable organisms in the atomizer are about equal, the *PR* allows direct comparison of the degradation of viability caused by the sampling processes of two collectors.

4.2.2 Stock Concentration

The concentration of the stock, C_s is evaluated as:

$$C_s = \frac{CFU_s \, a_r}{Q_s} \quad (4.5)$$

where

CFU_s is the total colony forming unit of the stock per *ml*

a_r is the rate of atomization of the Collison nebulizer (*ml*/min).

One possible method to get a more informed estimate of the aerosol output from our Collison nebulizer is to measure the liquid loss rate gravimetrically, then apply a correction factor (0.5~0.8) on the liquid loss rate to determine the atomization rate (Dennis *et al.,* 1992; Gatnash *et al.,* 1998; Ward *et al.,* 1998; Ward *et al.,* 1999; Young *et al.,* 1977) .

4.2.3 Atomization Rate

The atomization rate of the Collison nebulizer, a_r is estimated as:

$$a_r = L_r F_c \quad (4.6)$$

where

L_r is the liquid loss rate measured experimentally as 0.30 ml/min with 0.02 ml/min standard deviation.

F_c is a correction factor for the liquid loss rate based on the volume of the expended solution and the evaporation rate, (Young et al 1977) ranging from 0.5 to 0.8. In our calculations F_c was taken as the mean of 0.5 and 0.8 (0.65). The uncertainty was calculated with the extreme 0.5 and 0.8 values..

4.2.4 Aerosol to Hydrosol Collection Efficiency

The aerosol to hydrosol collection efficiency can be determined using the following equation:

$$\eta_{AH} = \frac{C_{m,air,hydrosol}}{C_{m,air,reference}} \quad (4.7)$$

where,

$C_{m,air,hydrosol}$ is the aerosol concentration based on fluorometric reading of hydrosol sample.

$C_{m,air,reference}$ is the aerosol concentration based on fluorometric reading from the reference sample.

The aerosol concentration of fluorescent dye in the sampled air, as calculated from analysis of the fluorescence of a solution was:

$$C_{m,air} = \frac{FV}{tQ} \quad (4.8)$$

where

$C_{m,air}$ is relative mass concentration of the fluorescent tracer in the sampled air

F is the numerical reading of the fluorometer

V is the solution volume

t is the time for a test

Q is the air flow rate.

4.3 Analysis of Variance (ANOVA)

The Analysis of Variance (or ANOVA) is a powerful and common statistical procedure in the social sciences. It can handle a variety of situations. Additionally, it is a powerful technique for analyzing experimental data involving quantitative measurements. It is particularly useful in factorial experiments where several independent sources of variation may be present (Lipson, 1973). For more details see

appendix C. In this study, unless otherwise indicated, the confidence level is taken to be 95%.

CHAPTER V

RESULTS AND DISCUSSION

5.1 *Bacillus globigii*

5.1.1 Survivability of the BG Spores Collected With a WWC Using 30% EG and 0.01% Tween-20 Collection Fluids, With and Without 0.5% OA

The BG samples analyzed indicated survivability between 64% and 119%, with the lowest value obtained for the samples in TW-20 at RT, (Table A.1, Figure B.10a and Figure B.10b).

At 99.9% confidence level using ANOVA statistical analysis, the addition of 0.5% OA to the collection fluid significantly increased the survivability of the BG in 30% EG at RT during the archiving period. However, there was no significant effect in EG samples archived at 4°C. In 0.01% TW-20, adding OA significantly increased the survivability at both temperatures with 99.9 % confidence levels.

The survivability in Tween-20, especially at RT significantly decreases, which is likely due to the surfactant effect of the Tween-20 in removing the spore coating and initiating germination (Hamoen and Errington, 2003). However, adding OA to both EG and TW-20 at RT samples significantly increased the survivability at the 99.9%

confidence level using the ANOVA statistical analysis. No significant effect was detected while the addition of OA to both EG and Tween-20 at 4°C samples.

Additionally, ANOVA statistical analysis indicated that changing the temperature significantly affected the survivability of BG samples in Tween-20, and a temperature of 4^{o}C provided a higher survivability with a 99.9% confidence; but, there was no significant effect indicated for the presence of OA in the Tween-20 samples. Also, changing the temperature had no significant effect in EG samples with or without the presence of OA.

5.1.2 Survivability of the BG Spores Collected With a WWC Using 30% EG and 0.01% Tween-20 Collection Fluids, With Antifoam B Added to the OA Suspensions

The samples analyzed showed between 25% and 113% survivability, with the lowest value obtained for the samples in TW-20 at RT (Table A.2 and Figure B.11a and Figure B.11b).

At the 99.9% confidence level, the archiving period had no significant effect on the survivability of BG in the samples except for the decrease in EG. However, adding OA to samples significantly increased the survivability among the day's especially at RT samples in both TW-20 and EG. A silica polymer that exists in the Antifoam B (A-F) in samples probably was the cause of the use of OA resulting in a significant increase in the survivability of BG, especially in EG samples at both temperatures.

Changing the temperature significantly affected the survivability of BG samples with at least 99% confidence, except in EG + OA + A-F, there was no significant effect indicated.

To investigate the effect of adding Antifoam B in the recovery of the spores, Figures B.12a and Figure B.12b shows the data conducted when the Antifoam B was in used without the ovalbumin. The statistical results showed with at least 99.5% confidence that adding Antifoam B increased the survivability in both EG and TW-20 samples at 4^{o}C. However, there was no significant difference indicated in the survivability at RT samples. Similar results were obtained considering the archiving period.

5.1.3 Survivability of the BG Spores Collected With an SKC Impinger Using 30% EG and 0.01% Tween-20 Collection Fluids, With and Without 0.5% OA

The samples showed between 29% and 114% survivability with the lowest value obtained for the samples collected in TW-20 and stored at RT, (Table A.3, Figure B.13a and Figure B.13b).

Adding OA to the EG samples at RT had an adverse effect on the survivability of BG samples at a confidence level of 99.9%. However, no significant effect was detected when OA was added to the EG samples at 4^{o}C. Oppositely, adding OA to the TW-20 samples significantly increased the survivability at both temperatures with at least 95% confidence level.

Regarding the effect of the archiving period, the archiving days had no significant effect on the survivability of BG except for the combination of OA added to EG. Changing the temperature significantly affected the survivability of BG samples in the collection fluids with at least 97.5% confidence, except in samples containing EG which indicated more stable survivability during the archiving period.

5.1.4 Survivability of the BG Spores Collected With an SKC Impinger Using 30% EG and 0.01% Tween-20 Collection Fluids, With Antifoam B Added to the OA

The results indicate between 11% and 100% survivability, with the lowest value associated with samples collected TW-20 at RT, (Table A.4, Figure B.14a and Figure B.14b).

Adding OA + A-F to the Tween-20 collection fluid significantly increased the survivability at both temperatures, and adding it to the EG solution increased survivability at RT, however, there was no significant effect indicated on the survivability of the BG in 30% EG at 4^{o}C.

Over the archiving period, the survivability significantly changed for the TW-20, EG and EG + OA + A-F. No significant effect was detected in TW-20 + OA + A-F.

Changing the temperature significantly affected the survivability of BG samples in both TW and EG with at least 99.5%. No significant effect was detected while using TW-20 + OA + A-F and EG + OA + A-F.

Figures B.15a and Figure B.15b shows the results when the Antifoam B was added to the fluid with ovalbumin. Adding A-F significantly increased the survivability

in Tween-20 samples; however, the opposite behavior was indicated while adding A-F to EG samples.

5.1.5 Comparison of the WWC and the SKC BioSampler

Figure B.16a presents the viability transfer ratio for the 100 LPM WWC and the 12.5 LPM SKC BioSampler as collected (day one), which clearly shows that the *VTR* of samples collected with the 100 LPM WWC is higher than samples collected with the 12.5 LPM SKC BioSampler. Additionally, Table A.5 shows the geometric mean of the performance ratio which calculated to be 1.51 with a standard deviation of 0.82.

Figure B.16b show the survivability obtained in the WWC and the SKC at the end of the archiving (day five). The statistical results indicated that the best collection fluid that can be used in the 100 LPM WWC in order to maintain the survivability of the collected BG during an archiving period of four days is EG + A-F, compared to EG in the SKC BioSampler.

5.2 *Escherichia coli*

5.2.1 Survivability of the *E. coli* Collected With a WWC Using 30% EG and 0.01% Tween-20 Collection Fluids, With and Without 0.5% OA

The samples showed survivability results between 65% and 114%, (Table A.6, Figure B.17a and Figure B.17b). The addition of OA to the collection fluid significantly

increased the survivability of the *E. coli* in 30% EG at both temperatures, (> 99.5% confidence level) during the archiving period. However it has been noted that adding OA had no significant effect on the survivability of the BG in TW-20 samples archived at both temperatures. The samples amended with OA survived longer as OA provides a protective coating on the bacteria surface and also a source of nutrients since *E. coli* is capable of binding ovalbumin mostly via heat shock proteins that are increasingly expressed by the bacteria in stress (Tobian *et al*., 2004).

The archiving period had no significant effect on the survivability of all the samples containing EG. The opposite behavior was observed in samples containing TW-20 especially without the presence of OA. As noted earlier, storage temperature significantly affected the survivability of BG samples in TW-20 and TW-20 + OA at the 95% confidence level.

5.2.2 Survivability of the *E. coli* Collected With an SKC Impinger Using 30% EG and 0.01% Tween-20 Collection Fluids, With and Without 0.5% OA

The samples showed between 56% and 100% survivability, (Table A.7, Figure B.18a and Figure B.18b). Adding OA to the TW-20 samples at RT and at 4^{o}C had an adverse effect (99.5% confidence level). However, the opposite behavior was indicated when OA was added to EG samples at both temperatures.

The archiving time had no significant effect on the survivability of *E. coli* in TW-20 and EG + OA. Changing the temperature significantly affected the survivability of *E.*

coli samples in TW-20, with 95% confidence, but, there was no significant effect for the other fluid compositions.

5.2.3 Comparison Between the WWC and the SKC BioSampler

Figure B.19a presents the viability transfer ratios of the 100 LPM WWC and the 12.5 LPM SKC BioSampler as collected (day one) for *E. coli*, which clearly shows that the *VTR* of samples collected with the 100 LPM WWC is higher than samples collected with the 12.5 LPM SKC BioSampler. Additionally, Table A.8 shows the geometric mean of the performance ratio which calculated to be 2.60 with a standard deviation of 0.16.

Figure B.19b shows the survivability obtained in the WWC and the SKC at the end of the archiving (day five). The statistical results indicated that the best collection fluid that can be used in the 100 LPM WWC in order to maintain the survivability of the collected *E. coli* during an archiving period of four days is EG + OA, compared to Tween-20 in the SKC BioSampler.

5.3 Aerosol to Hydrosol Collection Efficiency

Figure A.20 presents the aerosol to-hydrosol collection efficiency for the 100 LPM cyclone and the SKC BioSampler used in the experiments. The efficiency was 20% for 1μm AD particle size in the cyclone verses 70% in the SKC BioSampler when Tween-20 was used as the collection fluid.

CHAPTER VI

SUMMARY AND FURTHER WORK

In general after four days of storage, samples archived at 4˚C showed higher survivability than archived in RT. Additionally, Tween-20 significantly decreased the survivability during the archiving especially at RT. Furthermore, the survivability of the samples archived using EG as the collection fluid were more stable than that archived in Tween-20 in both collectors especially in BG spores, very likely due to the surfactant effect of the Tween-20, which removes the spore coat and initiates germination.

In the 100 LPM WWC, the addition of 0.5% ovalbumin to the collection fluid significantly increases the survivability of BG in the 30% EG and in 0.01% Tween-20 especially at RT. The same behavior was noticed in the 30% EG for the *E. coli* samples, suggesting that the addition of OA might be beneficial in maintaining the survivability in samples that are to be stored for a few days before analysis. Moreover, the statistical results showed that adding Antifoam B increased the survivability of BG in EG and in Tween-20 samples at 4°C, however there were no significant difference at RT. On the other hand, silica polymer represented in Antifoam B was added to the BG samples containing OA. The addition of silica polymer exists in the Antifoam B succeeded in increasing the survivability of BG in EG at both temperatures. In the SKC BioSampler, neither the addition of 0.5% ovalbumin nor 0.2% Antifoam B seems to have a beneficial

effect on the survivability of the BG spores in EG samples, however the opposite behavior was obtained in BG samples containing Tween-20.

Accordingly, the best collection fluid that can be used in the 100 LPM WWC in order to maintain the survivability of the collected BG is EG + A-F, compared to EG + OA in *E. coli* samples during an archiving period of four days. In the SKC BioSampler, the best collection fluid that can be used in order to maintain the survivability of the collected BG is EG. On the other hand, Tween-20 is the best collection fluid collecting *E. coli* samples during an archiving period of four days. This could be explained by the stabilizing effect of the EG on spores. However, at RT some of the spores will germinate and the EG has an adverse effect on vegetative cells due to its high osmotic pressure.

The viability transfer ratio of samples collected with the 100 LPM WWC is higher than samples collected with the 12.5 LPM SKC BioSampler for both *E. coli* and BG, very likely the WWC perform better than the SKC because the jet velocity in the SKC is sonic (about 300 m/s) while that for the WWC is about 23 m/s, and both have oblique impaction onto the collection surface. Accordingly, the geometric mean of the performance ratio calculated to be more than one, 1.51 with a standard deviation of 0.83 for BG samples and 2.60 with a standard deviation of 0.16 for *E. coli.*

Further methods are needed to determine the total number of particles, including both viable and dead in order to give a better estimate about the atomization rate. Concerning the requirements, new analysis methods such as polymerase chain reaction (PCR) and Coulter Counter (CC) have been initiated to be compared to our traditional

methods (APS), allowing a fast and reliable identification or cell count in order to obtain the most reliable method for total particle counts.

REFERENCES

Akers, A. B., and Won, W. D. (1969). Assay of Living Airborne Microorganisms. *In An Introduction to Experimental Aerobiology*, edited by R. L. Dimmick, and A. B. Akers. John Wiley and Sons, Inc., New York.

Black, R.S., and Shaw, M.J. (2002). Presentation at the Scientific Conference on Obscuration and Aerosol Research. U.S. Army Research, Development and Engineering Command/Edgewood Chemical Biological Center, Edgewood, MD.

Brachman, P. S., Ehrlich, R., Eichenwald, H. F., Gabelli, V. J., Kethley, T. W., and Madin, S. H. (1964). Standard Sampler for Assay of Airborne Microorganisms, *Science.* 144: 1295.

Buck, J. D. (1979). The Plate Count in Aquatic Microbiology. In *Native Aquatic Bacteria*, edited by J. W. Costerton, and R. R. Colwell. ASTM, Philadelphia.

Chatigny, M. A., Macher, J. M., Burge, H. A., and Solomon, W. R. (1989). Sampling Airborne Microorganisms and Aeroallergens. In *Air Sampling Instruments for Evaluation of Atmospheric Contaminants*, edited by S. V. Hering. ACGIH, Cincinnati, OH.

Daly, S. M., Przybycien, T. M., and Tilton, R. D. (2005). Adsorption of Poly (Ethylene Glycol) Modified Lysozyme to Silica, *Langmuir.* 21:1328-1337

Dennis, J. H., Avery, A. J., Walters, E. H. and Hendrick, D. J. (1992). Calibration of Aerosol Output From the Mefardosimeter- Implications for Epidemiologic. *Eur. Resp. J.* 5:1279-1282.

Errington, F. P., and Powell, E. O. (1969). A Cyclone Separator for Aerosol Sampling in the Field, *J. Hyg. Camb.* 67:387-399.

Gatnash, A. A., Chandler, S. T., and Connolly, C. K. (1998). A New Method for Measuring Aerosol Nebulizer Output Using Radioactive Tracers. *Eur. Resp. J.* 12:467-471.

Griffiths, W. D., and DeCosemo, G. A. (1994). The Assessment of Bio-aerosols: *A Critical Review*, *J. Aerosol Sci.* 25:1425-1458.

Grinshpun, S. A., Willeke, K., Ulevicius, V., Juozaitis, A., Terzieva, S.,Donnelly, J., Stelma, G. N., and Brenner, K. P. (1997). Effect of Impaction, Bounce and Reaerosolization on the Collection Efficiency of Impingers, *Aerosol Sci. Technol*. 26:326- 342.

Hamoen, L. W., and Errington, J. P. (2003). Targeting of DivIVA in *Bacillus subtilis* Is Not Directly Dependent on FtsZ or PBP 2B, *Journal of Bacteriology*. 185(2):693-697.

Henningson, E. W., and Ahlberg, M. S. (1994). Evaluation of Microbiological Aerosol Samplers*: A Review*, *J. Aerosol Sci.* 25:1459-1492.

Henningson, E. W., Fangmark, I., Larsson, E., and Wikstrom, L. E. (1988). Collection Efficiency of Liquid Samplers for Microbiological Aerosols, *J. Aerosol Sci.* 19:911-914.

Hinds, W. C. (1999). *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles.* 2nd Edition. John Wiley and Sons, Inc. New York.

Jensen, P. A., and Schafer, M. (1998). *Sampling and Characterization of Bio-aerosols,* NIOSH Manual of Analytical Methods. pp.82-112. www.cdc.gov/niosh/nman.

Jensen, P. A., Todd, W. F., Davis, G. N., and Scarpino, P. V. (1992). Evaluation of Eight Bio-aerosol Samplers Challenged With Aerosols of Free Bacteria, *Am. Ind. Hyg. Assoc.* 53:660-667.

Jones, J. G. (1979). Direct Counts on Membrane Filters. In *A Guide to Methods for Estimating Microbial Numbers and Biomass in Fresh Water*, *Freshwater Biology Association Sci.* Titus Wilson & Son Ltd, Ambleside. 39:19-39.

Lehtimaki, M., and Willeke, K. (1993). *In Measurement Principles Techniques and Applications*, edited by K. Willeke, and P. A. Baron. van Nostrand Reinhold. New York.

Lembke, L. L., Kniseley, R. N., Nostrand, R.V., and Hale, M.D. (1981). Precision of the All-Glass Impinger and the Andersen Microbial Impactor for Air Sampling in Solid-Waste Handling Facilities, *Appl. Environ. Microbiol.* 42:222-225.

Lin, X., Reponen, T., Willeke, K., and Wang, Z. (2000). Survival of Airborne Microorganisms During Swirling Aerosol Collection, *Aerosol Sci. Technol*. 32:184-196.

Lin, X., Willeke, K., Ulevicius, V., and Grinshpun, S. A. (1997). Effect of Sampling Time on the Collection Efficiency of All-Glass Impingers, *Am*. *Ind*. *Hyg*. *Assoc*. *J*. 58:480-488.

Lipson, C., and Sheth, N.J. (1973). *Statistical Design and Analysis of Engineering Experiments.* McGraw-Hill Book Company, New York.

Macher, J. M., and Willeke, K. (1993). Performance Criteria for Bio-aerosol Samplers, *J. Aerosol Sci*. 23:S647-S650.

Macher, J. M., Chatigny, M. A., and Burge, H. A. (1995). Sampling Airborne Microorganisms and Aeroallergens. In *Air Sampling Instruments for Evaluation of Atmospheric Contaminants*, edited by B. S. Cohen, and S. V. Hering, 8th ed., ACGIH. Cincinnati, OH.

McFarland , A. R., Haglund, J. S., King, M. D., Hu, S., Phull, M. S., Moncla, B. W., and Seo, Y. (2009). Wetted Wall Cyclones for Bioaerosol Sampling, *Aerosol Sci. Technol*. In Submission.Moncla, B. (2004). *A Study of Bio-aerosol Sampling Cyclones*. M.S. Thesis, Department of Mechanical Engineering, Texas A&M University, College Station, TX.

Phull, M. (2005). An Improved Wetted Wall Bio-aerosol Sampling Cyclone. M.S. Thesis, Department of Mechanical Engineering, Texas A&M University, College Station, TX.

Robison, R. L., and Weinswig, M. H. (1968). Effect of Polyethylene Glycol 300 on the Viability of Bacterial Spores, *Journal of Pharmaceutical sciences*. 58(2):275-276.

Salusbury, T. T., Deans, J. S. and Stewart, I. W. (1988). A Comparison of Six Devices Used to Sample Aerosols of *Bacillus subtilis* var. *niger* Spores. *Proc. Aerosol Soc. 2nd conferecnce*. pp. 101-105.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Seo, Y. (2007). *Design of Wetted Wall Bio-aerosol Concentration Cyclone*. PhD. Dissertation, Department of Mechanical Engineering, Texas AandM University, College Station, TX.

Sneath, P. H. (1986). Endospore-Forming Gram-positive Rods and Cocci, edited by P. H. A. Sneath, N. S. Mari, M. E. Shape. *In Bergey' s Manual of Systematic Bacteriology*. 2:1104-1139.

Srikanth, P., Sudharsanam, S., and Steinb, R. (2008). Bio-aerosol in Indoor Environment: *Composition, Health Effect and Analysis*, *Indian Journal of Medical Microbiology*. 26(4):302-312.

Sun, N., Wang, H., Chen, Y., Lu, S., and Xiong, Y. (2008). Effect of Surfactant SDS, Tween 80, Triton X-100 and Rhamnolipid on Biodegradation of Hydrophobic Organic Pollutants, IEEE. pp. 4730-4734.

Tobian, A.A.R., Canaday, D.H., and Harding, C. V. (2004). Bacterial Heat Shock Proteins Enhance Class II MHC Antigen Processing and Presentation of Chaperoned Peptides to $CD4^{+}$T Cells, *J. Immunol.* 173:5131-5137.

Tortora, G. J., Funke, B. R., and Case, C. L. (1989). *Microbiology: An Introduction.* 3rd., ed. Benjamin/Cummings. Redwood City, CA.

Ward, R. J., Liakakos, P., Leonard, R. F., Reid, D. W., Johns, D. P., and Walters, E. H. (1999). A Critical Evaluation of the Mefar (TM) Dosimeter. *Eur. Resp. J.* 14:430-434.

Ward, R. J., Reid, D. W., Leonard, R. F., Johns, D. P., and Walters, E. H. (1998). Nebulizer Calibration Using Lithium Chloride: An Accurate, Reproducible and User-Friendly Method. *Eur. Resp. J.* 11:937-941.

Wathes, C. M., and Randall, J. M. (1988). Aerosol Sampling in Animal Houses. Proceedings of a workshop held at the University of Bristol, 26-29 July, 1988. Commission of the European Communities, Luxembourg, Luxembourg

Wells, W. F. (1933). Apparatus for Study of Bacterial Behavior of Air, *Am. J. Public. Hth.* 23:58-99.

White, L.A., Hadley, D.J., Davids, D.E., and Naylor, R. (1975). Improved Large-volume Sampler for the Collection of Bacterial Cells from Aerosol, *Appl. Microbiology.* 29:335-339.

Willeke, K., Lin, X., and Grinshpun, S. A. (1998). Improved Aerosol Collection by Combined Impaction and Centrifugal Motion, *Aerosol Sci. Technol.* 28:439-456.

Young, H. W., Dominik, J. W., Walker, J. S. and Larson, E. W. (1977). Continuous Aerosol Therapy System Using a Modified Collsion. *J. Clin. Microbiol.* 5:131-136.

APPENDIX A

TABLES

Table A.1. Survivability of 100 LPM WWC collected BG samples during a five day period in 30% EG and 0.01% Tween-20, with and without OA at 4°C and RT. The values following the ± symbols are one standard deviation about the mean.

Period (Day)	4°C Survivability				RT Survivability			
	0.01% Tween-20	30% EG	0.01% Tween-20 + OA + A-F	30% EG + OA + A-F	0.01% Tween-20	30% EG	0.01% Tween-20 + OA + A-F	30% EG + OA + A-F
1	100 ± 4	100 ± 15	100 ± 9	100 ± 15	100 ± 4	100 ± 15	100 ± 9	100 ± 15
2	99 ± 8	100 ± 9	108 ± 3	113 ± 17	88 ± 7	106 ± 8	105 ± 11	106 ± 10
3	105 ± 3	119 ± 6	111 ± 9	110 ± 11	89 ± 5	104 ± 5	106 ± 7	110 ± 10
4	103 ± 7	117 ± 10	112 ± 16	108 ± 8	82 ± 6	97 ± 8	105 ± 19	105 ± 9
5	93 ± 1	99 ± 8	116 ± 4	96 ± 10	64 ± 5	96 ± 18	83 ± 18	102 ± 12

Table A.2. Survivability of 100 LPM WWC collected BG samples during a five day period in 30% EG and 0.01% Tween-20, with and without OA at 4°C and RT and with Antifoam B in the OA samples. The values following the ± symbols are one standard deviation about the mean.

Period (Day)	4°C Survivability				RT Survivability			
	0.01% Tween-20	**30% EG**	**0.01% Tween-20 + OA + A-F**	**30% EG + OA + A-F**	**0.01% Tween-20**	**30% EG**	**0.01% Tween-20 + OA + A-F**	**30% EG + OA + A-F**
1	100 ± 20	100 ± 25	100 ± 20	100 ± 1	100 ± 3	100 ± 25	100 ± 20	100 ± 1
2	100 ± 3	102 ± 19	102 ± 16	103 ± 15	90 ± 15	86 ± 11	84 ± 20	113 ± 8
3	94 ± 3	91 ± 4	96 ± 6	107 ± 13	65 ± 6	68 ± 12	73	98 ± 10
4	87 ± 28	82 ± 15	84 ± 14	92 ± 15	32 ± 7	69 ± 18	76	97 ± 9
5	78 ± 14	70 ± 6	85 ± 11	101 ± 17	25 ± 9	49 ± 13	69	91 ± 6

Table A.3. Survivability of 12.5 LPM SKC Impinger collected BG samples during a five day period in 30% EG and 0.01% Tween-20, with and without OA at 4°C and RT. The values following the ± symbols are one standard deviation about the mean.

Period (Day)	4°C Survivability				RT Survivability			
	0.01% Tween-20	**30% EG**	**0.01% Tween-20 + 0.5% OA**	**30% EG + 0.5% OA**	**0.01% Tween-20**	**30% EG**	**0.01% Tween-20 + 0.5% OA**	**30% EG + 0.5% OA**
1	100 ± 10	100 ± 21	100 ± 18	100 ± 11	100 ± 10	100 ± 21	100 ± 18	100 ± 11
2	90 ± 10	103 ± 5	97 ± 7	105 ± 6	55 ± 2	102 ± 12	81 ± 7	92 ± 13
3	94 ± 16	95 ± 12	103 ± 3	114 ± 22	56 ± 1	103 ± 5	85 ± 3	103 ± 16
4	82 ± 5	97 ± 11	89 ± 6	92 ± 15	48 ± 3	101 ± 8	76 ± 9	90 ± 2
5	85 ± 2	97 ± 3	94 ± 1	99 ± 5	29 ± 7	100 ± 9	77 ± 4	101 ± 12

Table A.4. Survivability of 12.5 LPM SKC Impinger collected BG samples during a five day period in 30% EG and 0.01% Tween-20, with and without OA at 4°C and RT and with Antifoam B in the OA samples. The values following the ± symbols are one standard deviation about the mean.

Period (Day)	4°C Survivability				RT Survivability			
	0.01% Tween-20	**30% EG**	**0.01% Tween-20 + OA + A-F**	**30% EG + OA + A-F**	**0.01% Tween-20**	**30% EG**	**0.01% Tween-20 + OA + A-F**	**30% EG + OA + A-F**
1	100 ± 39	100 ± 14	100 ± 24	100 ± 22	100 ± 39	100 ± 14	100 ± 24	100 ± 22
2	76 ± 18	81 ± 13	65 ± 21	75 ± 17	63	93 ± 7	66 ± 12	95 ± 49
3	77	85 ± 1	89 ± 11	96 ± 8	39	82 ± 8	67 ± 15	90 ± 13
4	60	86 ± 14	78 ± 9	62 ± 13	18	82 ± 8	57 ± 15	60 ± 13
5	54	72 ± 4	64 ± 15	72 ± 15	11	67 ± 3	53 ± 9	64 ± 19

Table A.5. Performance ratio of BG samples collected with the WWC and the SKC as collected.

Collection Fluid	WWC VTR	WWC STDV	WWC Uncertainty	SKC VTR	SKC STDV	SKC Uncertainty	PR	Geo_Avg PR
TW-20	0.60	0.21	0.15	0.45	0.10	0.11	1.35	1.51±0.83
TW-20 + OA	0.49	0.04	0.12	0.44	0.08	0.11	1.10	
TW-20 + A-F	0.36	0.07	0.09	0.28	0.09	0.07	1.26	
TW-20+OA+ A-F	0.69	0.14	0.17	0.21	0.05	0.05	3.30	
EG	0.49	0.10	0.12	0.50	0.11	0.12	0.98	
EG + OA	0.57	0.09	0.14	0.47	0.05	0.12	1.21	
EG + A-F	0.81	0.09	0.20	0.57	0.19	0.14	1.42	
EG + OA + A-F	0.75	0.01	0.18	0.29	0.06	0.07	2.56	

Table A.6. Survivability of 100 LPM WWC collected *E. coli* samples during a five day period in 30% EG and 0.01% Tween-20, with and without OA at 4°C and RT. The values following the ± symbols are one standard deviation about the mean.

Period (Day)	4°C Survivability				RT Survivability			
	0.01% Tween-20	**30% EG**	**0.01% Tween-20 + 0.5% OA**	**30% EG + 0.5% OA**	**0.01% Tween-20**	**30% EG**	**0.01% Tween-20 + 0.5% OA**	**30% EG + 0.5% OA**
1	100 ± 9	100 ± 27	100 ± 11	100 ± 28	100 ± 9	100 ± 27	100 ± 11	100 ± 28
2	86 ± 16	87 ± 17	93 ± 17	98 ± 19	83 ± 12	87 ± 13	84 ± 16	113 ± 61
3	109 ± 21	99 ± 27	92 ± 27	114 ± 35	91 ± 15	92 ± 23	86 ± 11	98 ± 41
4	86 ± 12	108 ± 20	112 ± 20	114 ± 48	75 ± 14	93 ± 30	74 ± 22	109 ± 38
5	95 ± 28	91 ± 33	103 ± 33	97 ± 41	66 ± 11	88 ± 30	65 ± 16	96 ± 37

Table A.7. Survivability of 12.5 LPM SKC Impinger collected *E. coli* samples during a five day period in 30% EG and 0.01% Tween-20, with and without OA at 4°C and RT. The values following the ± symbols are one standard deviation about the mean.

Period (Day)	4°C Survivability				RT Survivability			
	0.01% Tween-20	**30% EG**	**0.01% Tween-20 + 0.5% OA**	**30% EG + 0.5% OA**	**0.01% Tween-20**	**30% EG**	**0.01% Tween-20 + 0.5% OA**	**30% EG + 0.5% OA**
1	100 ± 4	100 ± 7	100 ± 6	100 ± 10	100 ± 4	100 ± 7	100 ± 6	100 ± 10
2	88 ± 19	68 ± 8	72 ± 33	86 ± 21	89 ± 5	78 ± 9	78 ± 29	68 ± 10
3	90 ± 11	76 ± 9	79 ± 46	74 ± 4	85 ± 16	68 ± 6	92 ± 9	96 ± 7
4	94 ± 16	66 ± 1	82 ± 5	77 ± 2	83 ± 15	63 ± 6	86 ± 7	85 ± 11
5	89 ± 12	64 ± 10	57 ± 9	93 ± 10	76 ± 9	57 ± 4	56 ± 5	72 ± 2

Table A.8. Performance Ratio of *E. coli* samples collected with the WWC and the SKC as collected (day one).

Collection Fluid	WWC VTR	WWC STDV	WWC Uncertainty	SKC VTR	SKC STDV	SKC Uncertainty	PR	Geo_Avg PR
TW-20	0.48	0.03	0.12	0.20	0.01	0.05	2.37	2.60±0.16
TW-20 + OA	0.57	0.05	0.14	0.21	0.01	0.05	2.70	
EG	0.78	0.17	0.19	0.29	0.02	0.07	2.67	
EG + OA	0.73	0.17	0.18	0.27	0.02	0.07	2.66	

APPENDIX B

FIGURES

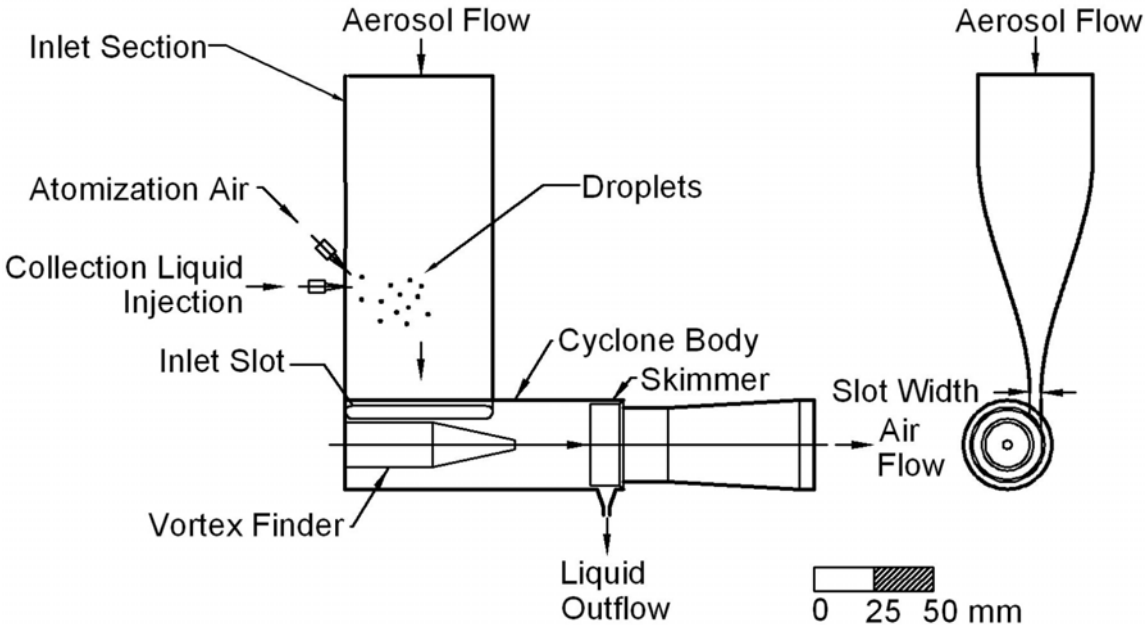

Figure B.1. Concept of wetted wall cyclone (McFarland, 2009)

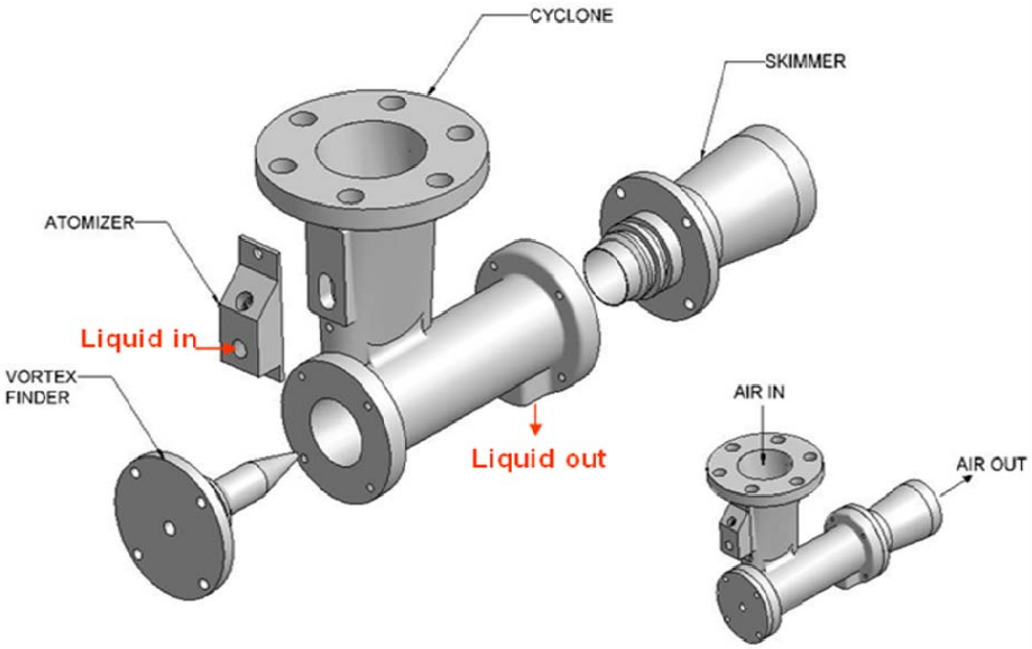

Figure B.2. Construction of the 100 LPM wetted wall cyclone (Seo, 2007)

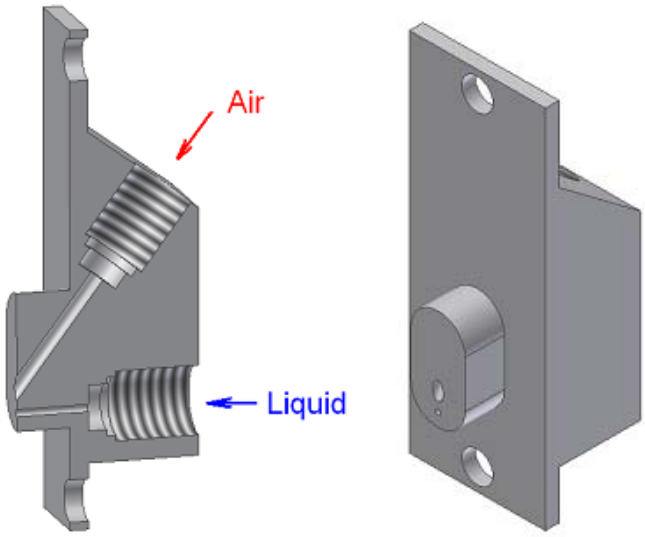

Figure B.3. The two needles of atomizer (for uniform dispersion)

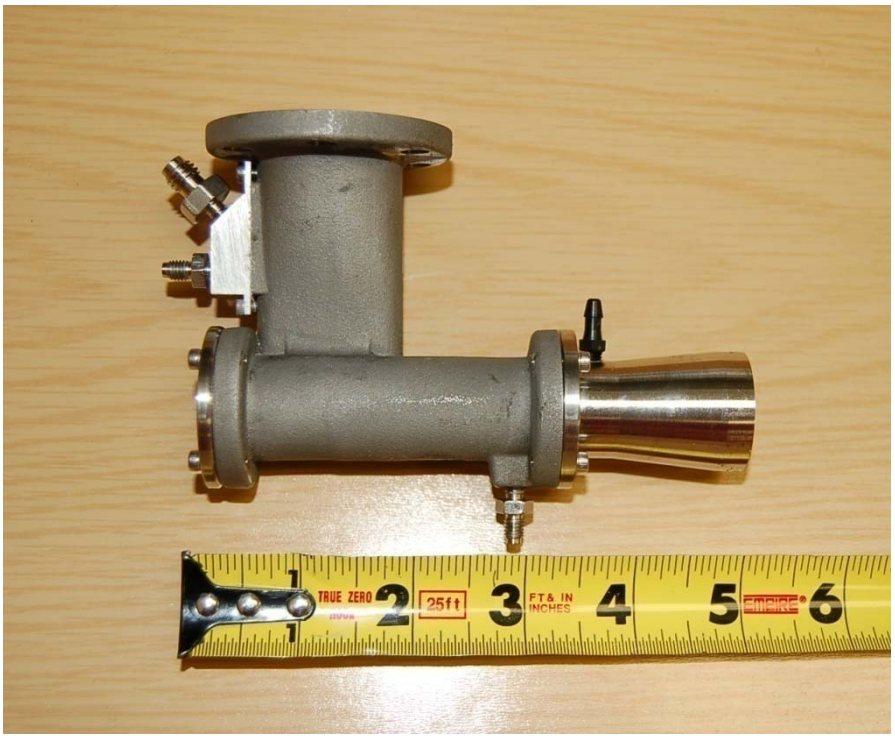

Figure B.4. The 100 LPM wetted wall cyclone unit used in this study

Figure B.5. SKC BioSampler Impinger used in this study

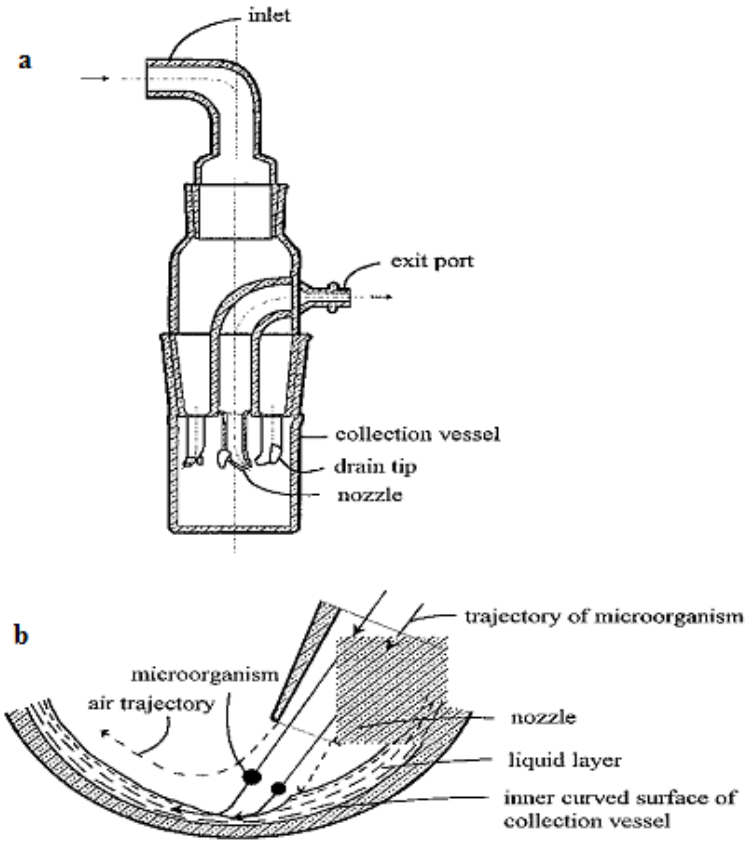

Figure B.6. The SKC BioSampler. a) schematic diagram; b) collection mechanism (Lin *et al*, 2000)

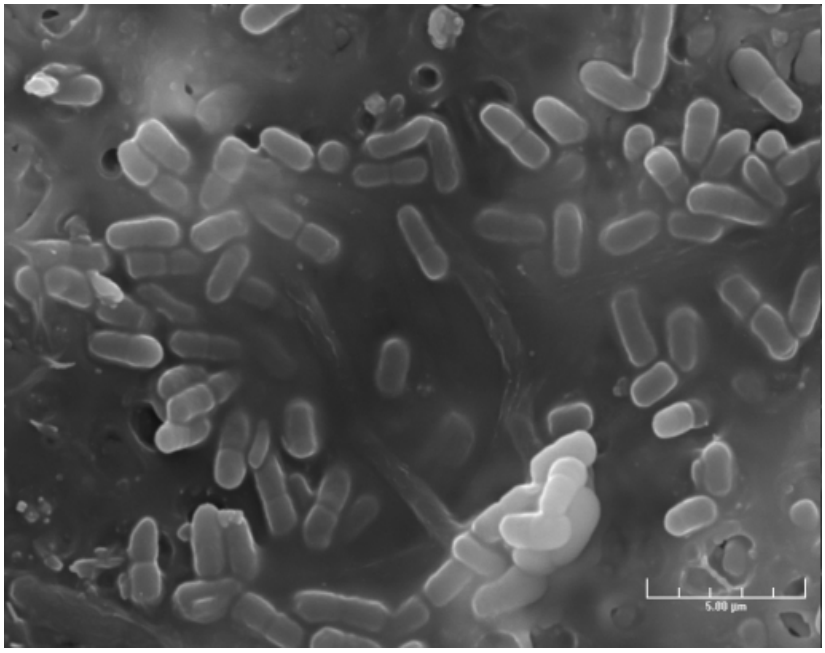

Figure B.7. Scanning Electron Microscopy image of fresh *E. coli* culture

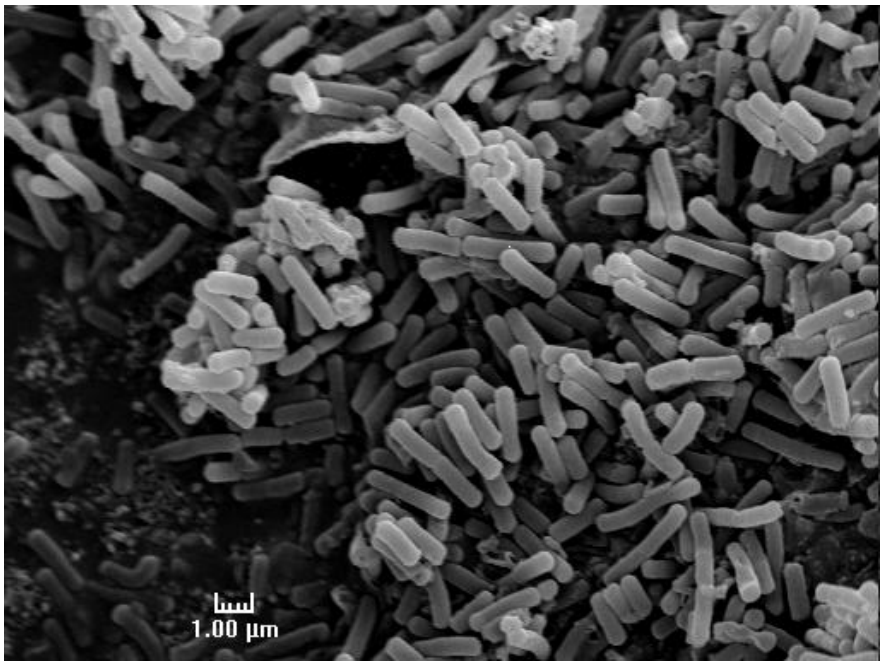

Figure B.8. Scanning Electron Microscopy image of sporulating *Bacillus globigii*

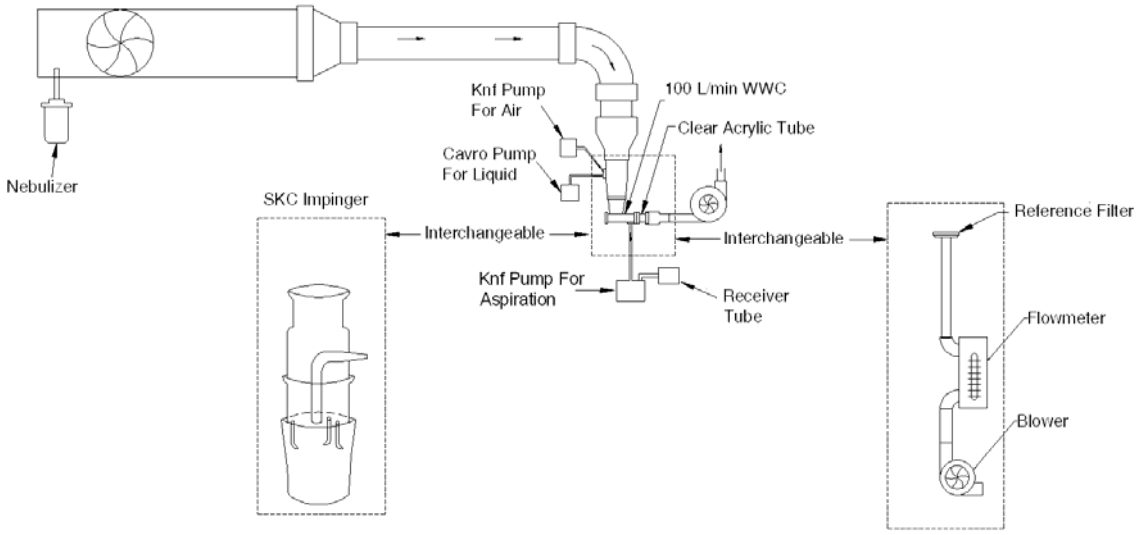

Figure B.9. Experimental setup

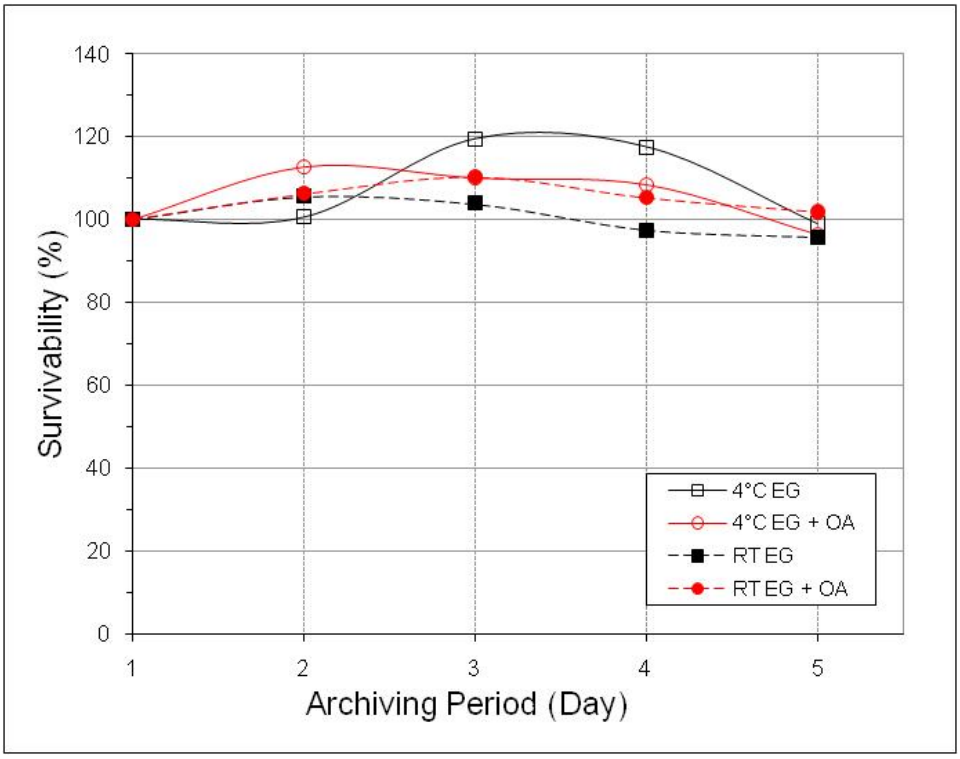

Figure B.10a. Survivability of 100 LPM WWC-collected BG samples during a five day period in 30% EG, with and without OA at 4^{o}C and RT. See Table A.1 for standard deviation values associated with each data point

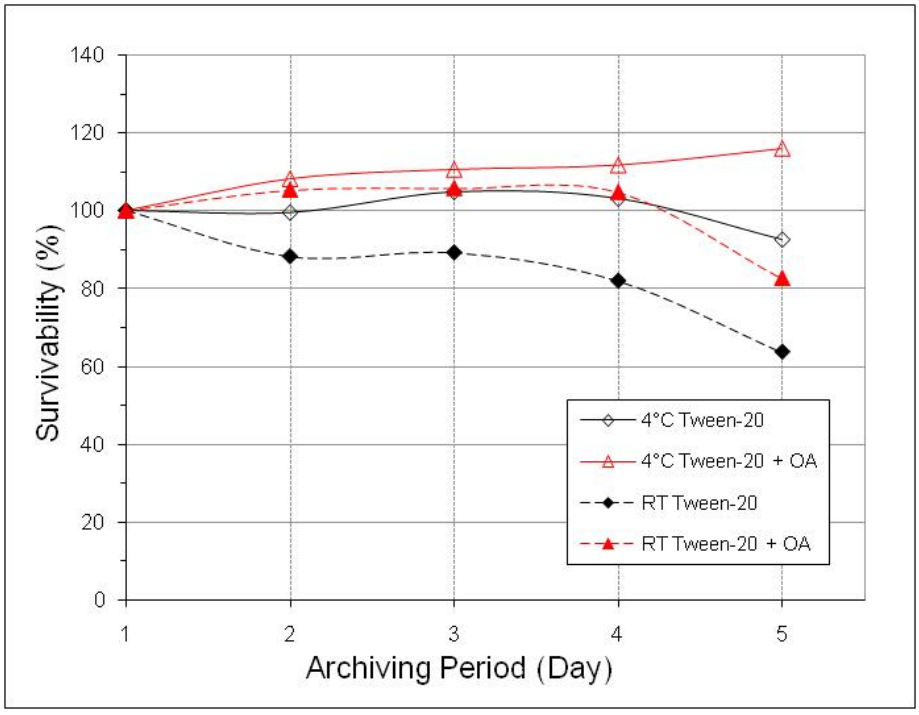

Figure B.10b. Survivability of BG samples collected with a 100 LPM WWC. Collection liquid was 0.01% Tween-20, with and without OA, and storage was at 4^{o}C and RT. See Table A.1 for standard deviation values associated with each data point

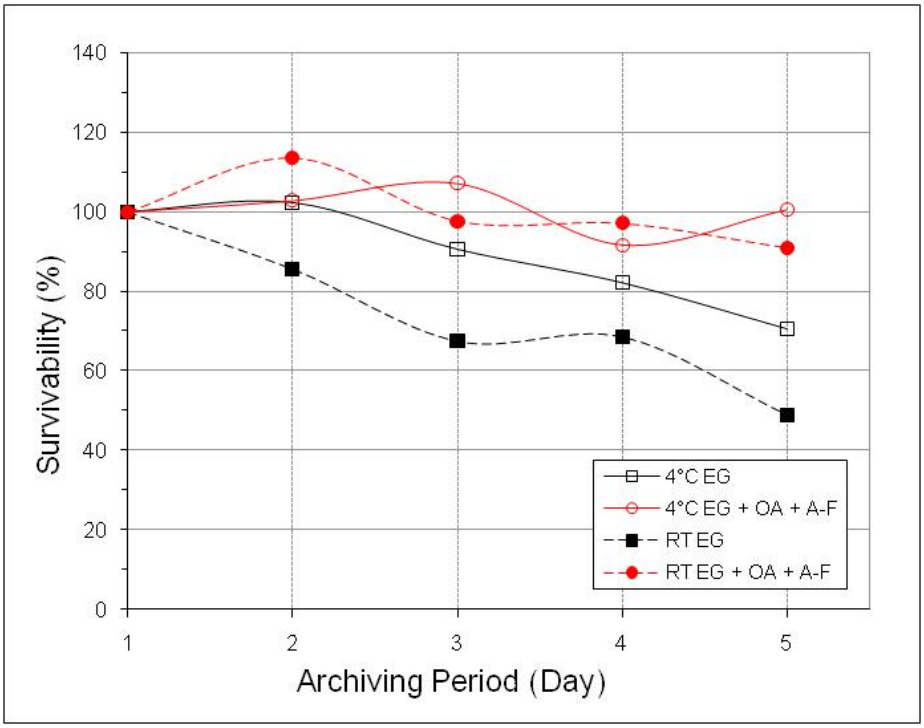

Figure B.11a. Survivability of 100 LPM WWC-collected BG samples during a five day period in 30% EG, with and without OA at 4°C and RT, with Antifoam B added to the OA samples. See Table A.2 for standard deviation values associated with each data point

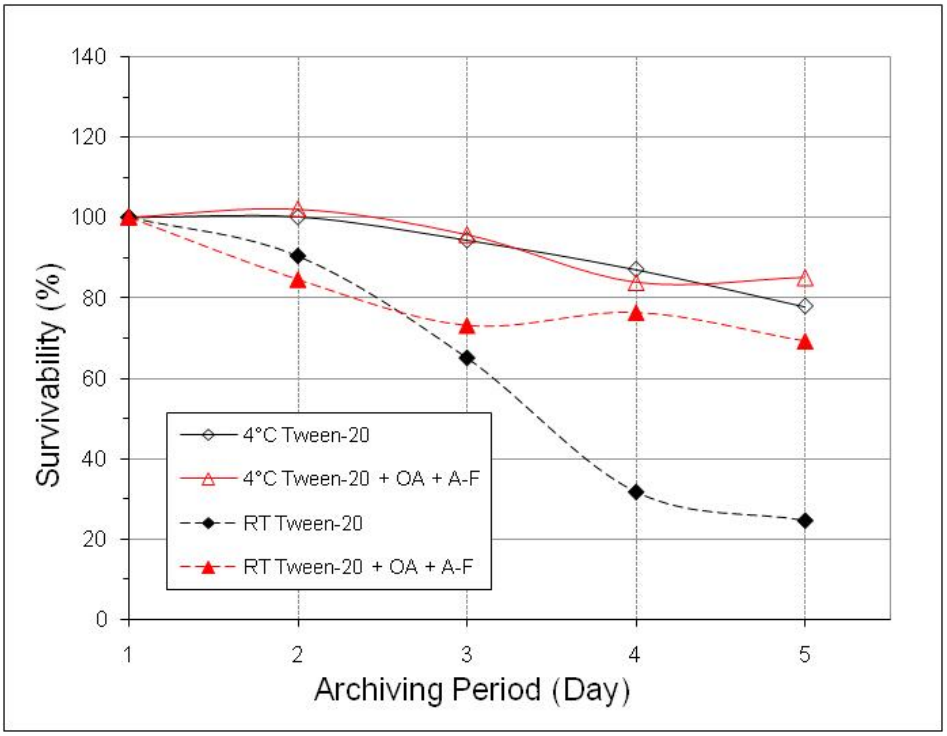

Figure B.11b. Survivability of 100 LPM WWC-collected BG samples during a five day period in 0.01% Tween-20, with and without OA at 4^{o}C and RT with the Antifoam B added to the OA samples. See Table A.2 for standard deviation values associated with each data point

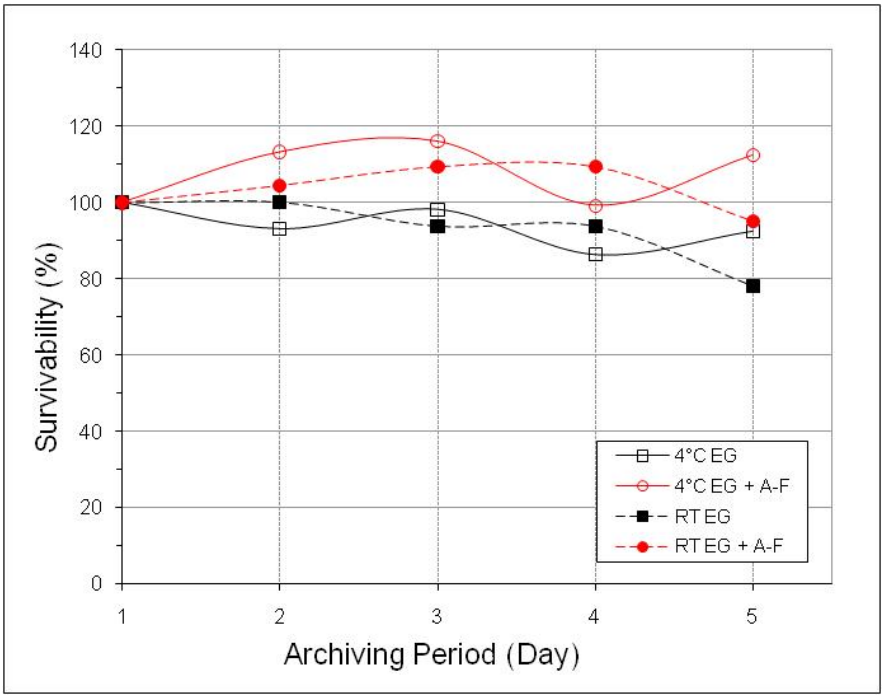

Figure B.12a. Survivability of 100 LPM WWC-collected BG samples during a five day period in 30% EG, with and without Antifoam B at 4^{o}C and RT

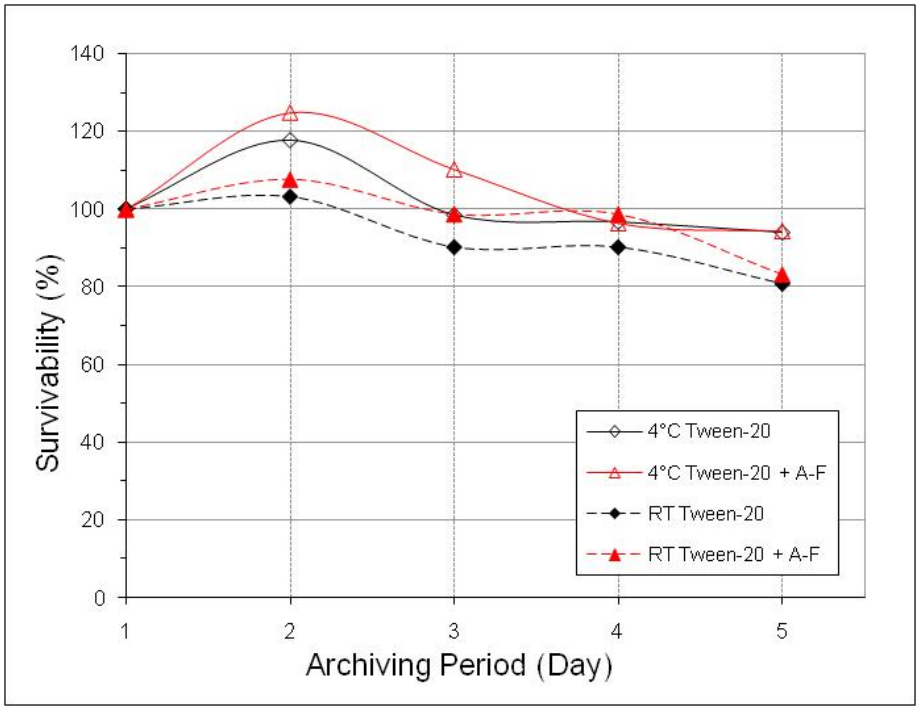

Figure B.12b. Survivability of 100 LPM WWC-collected BG samples during a five day period in 0.01% Tween-20, with and without Antifoam B at 4^{o}C and RT

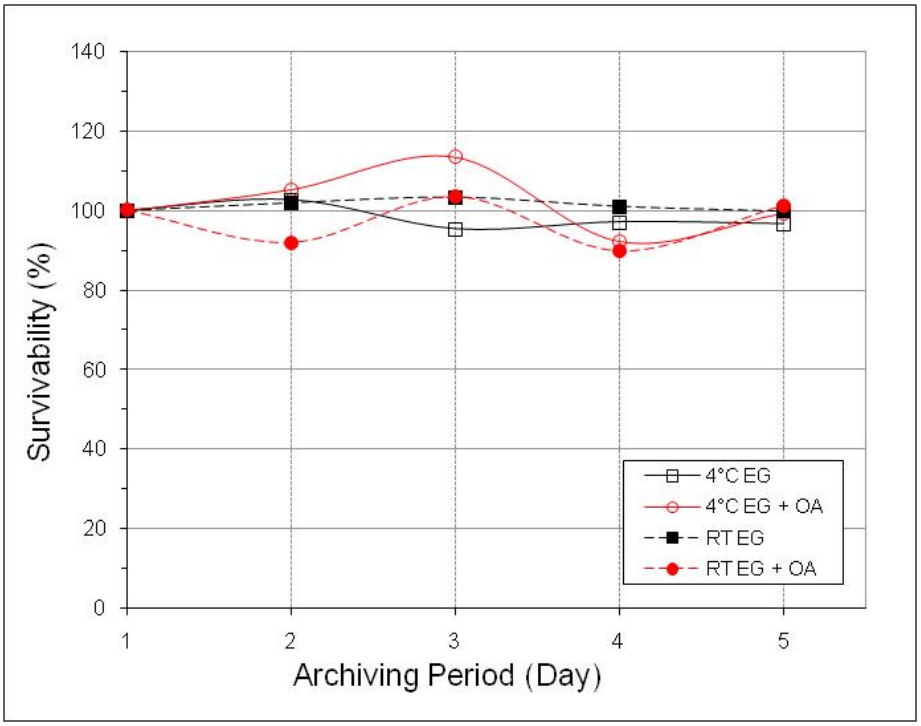

Figure B.13a. Survivability BG samples collected with a 12.5 LPM SKC Impinger. Collection fluid was 30% EG, with and without OA. Storage was at 4°C and RT See Table A.3 for standard deviation values associated with each data point

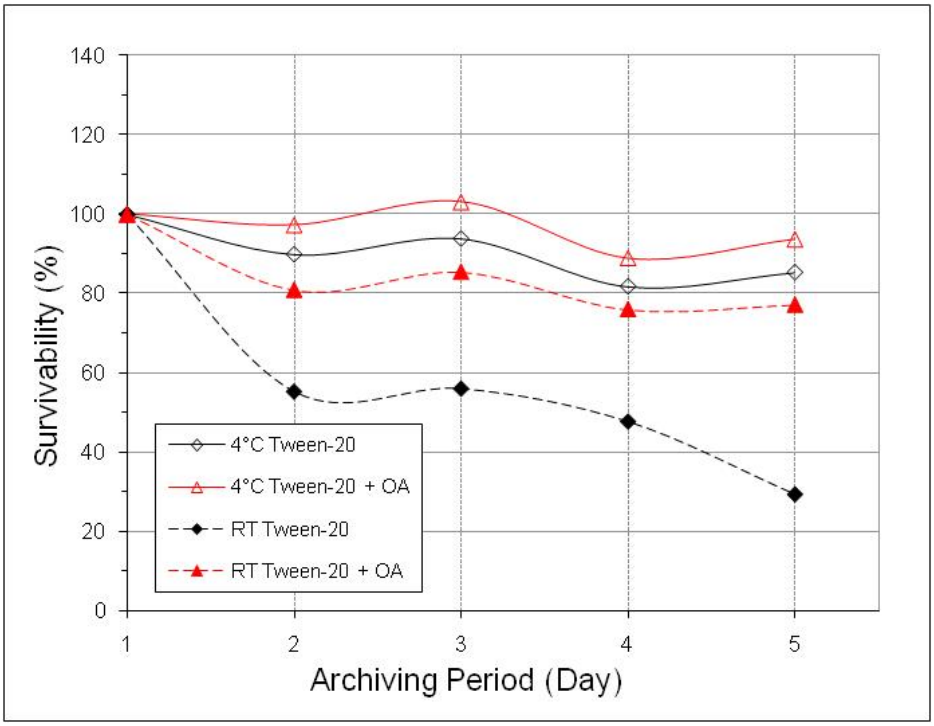

Figure B.13b. Survivability of 12.5 LPM SKC Impinger-collected BG samples during a five day period in 0.01% Tween-20, with and without OA at 4^{o}C and RT. See Table A.3 for standard deviation values associated with each data point

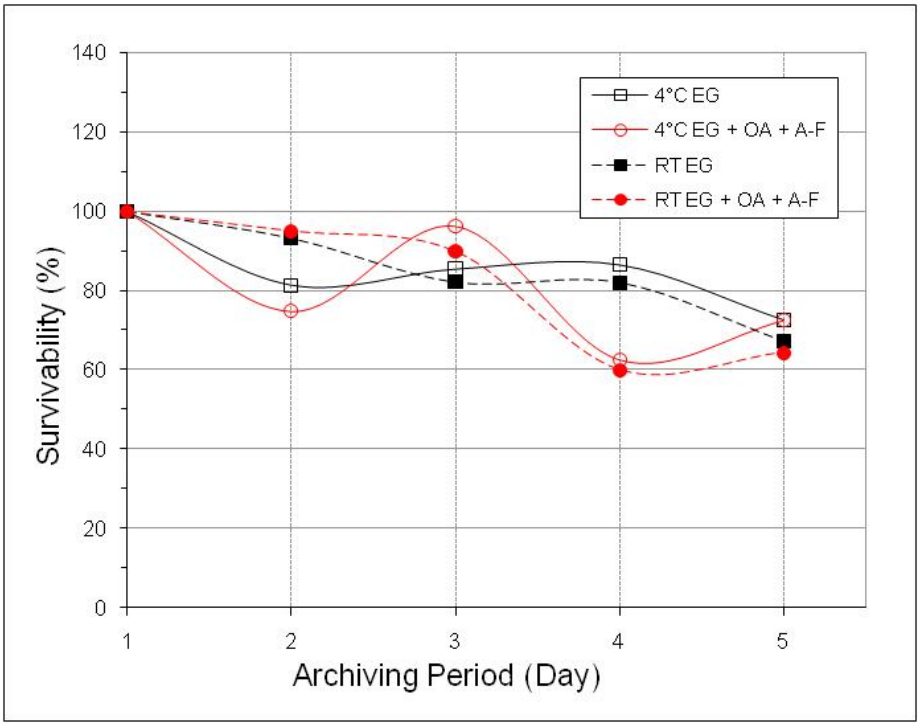

Figure B.14a. Survivability of 12.5 LPM SKC Impinger-collected BG samples during a five day period in 30% EG, with and without OA at 4°C and RT with Antifoam B in the OA samples. See Table A.4 for standard deviation values associated with each data point

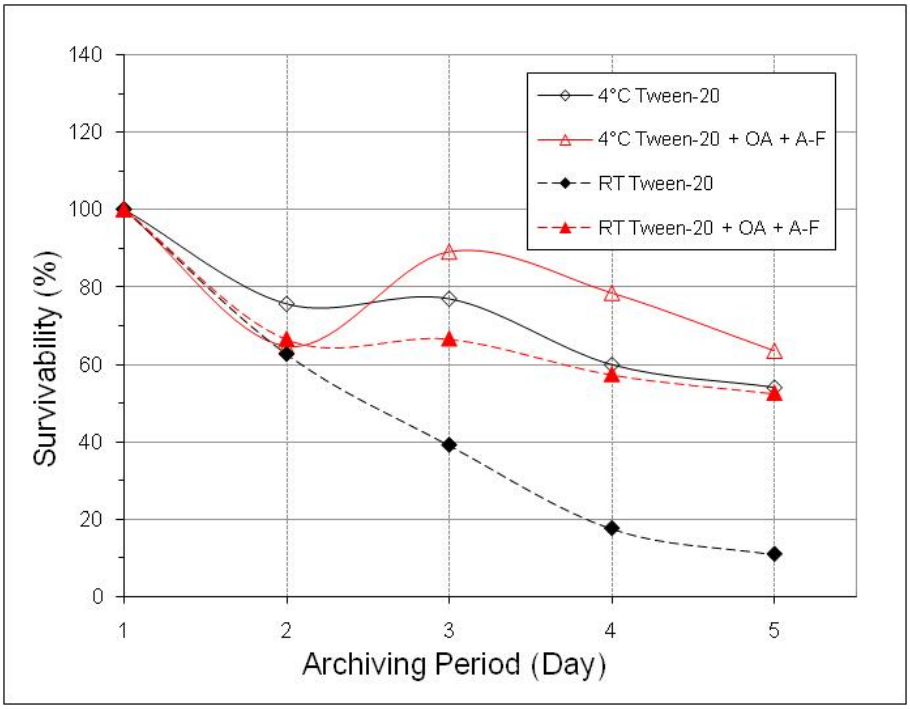

Figure B.14b. Survivability of 12.5 LPM SKC Impinger-collected BG samples during a five day period in 0.01% Tween-20, with and without OA at 4^{o}C and RT with Antifoam B in the OA samples See. Table A.4 for standard deviation values associated with each data point

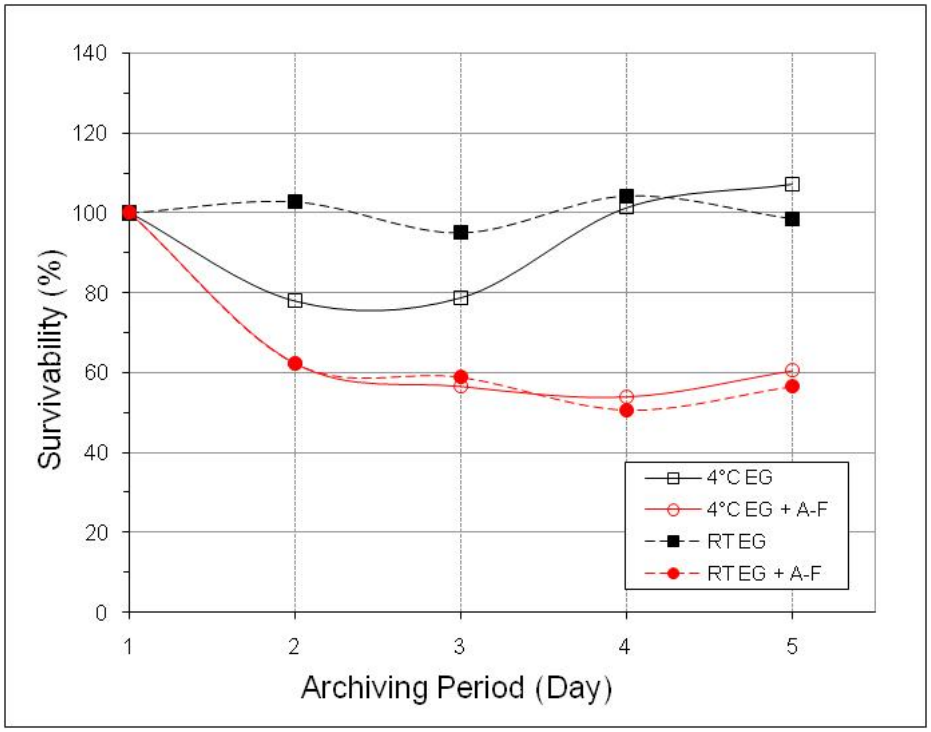

Figure B.15a. Survivability of 12.5 LPM SKC Impinger-collected BG samples during a five day period in 30% EG, with and without Antifoam B at 4^{o}C and RT

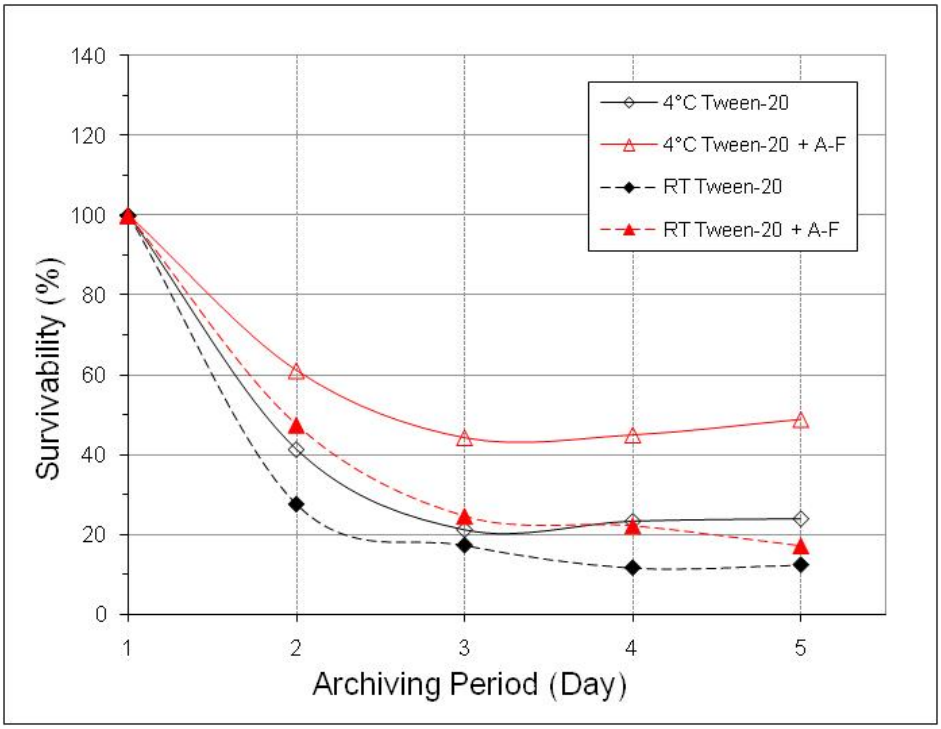

Figure B.15b. Survivability of 12.5 LPM SKC Impinger-collected BG samples during a five day period in 0.01% Tween-20, with and without Antifoam B at 4^{o}C and RT

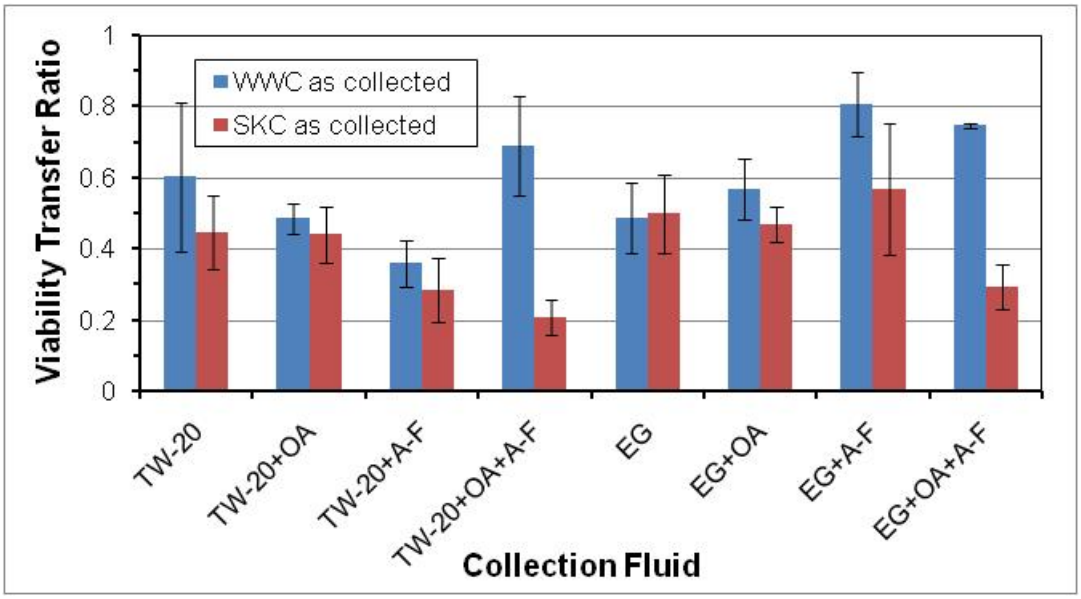

Figure B.16a. Comparison of the WWC and the SKC viability transfer ratio as collected (day one) for BG samples with the standard deviation shown in the same figure. See Table A.5 for the uncertainty values associated with each data point

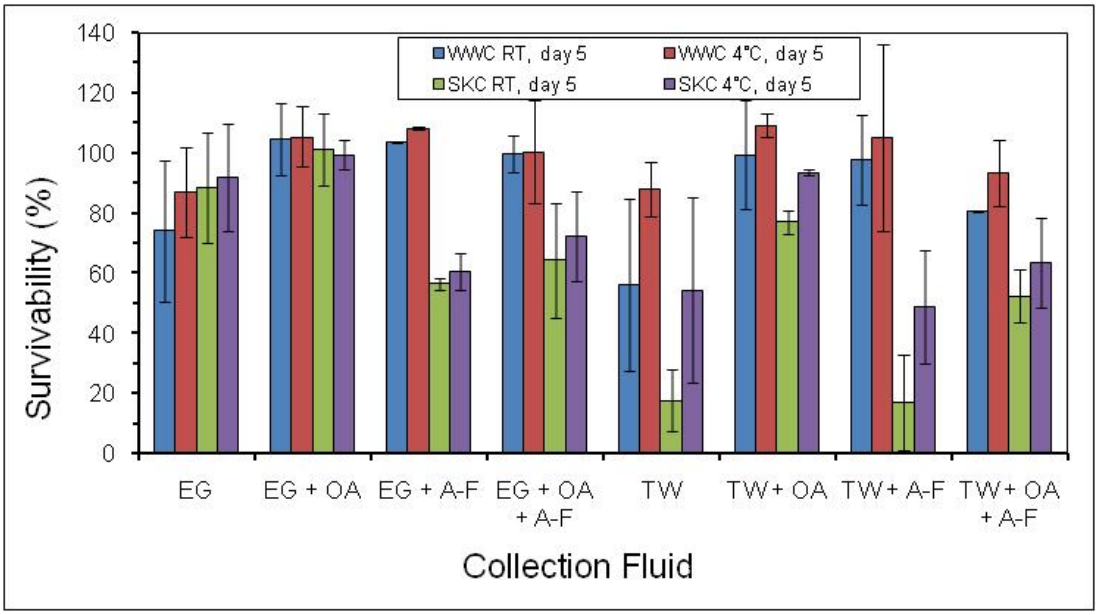

Figure B.16b. Survivability comparison on day five of BG samples collected with the WWC and the SKC

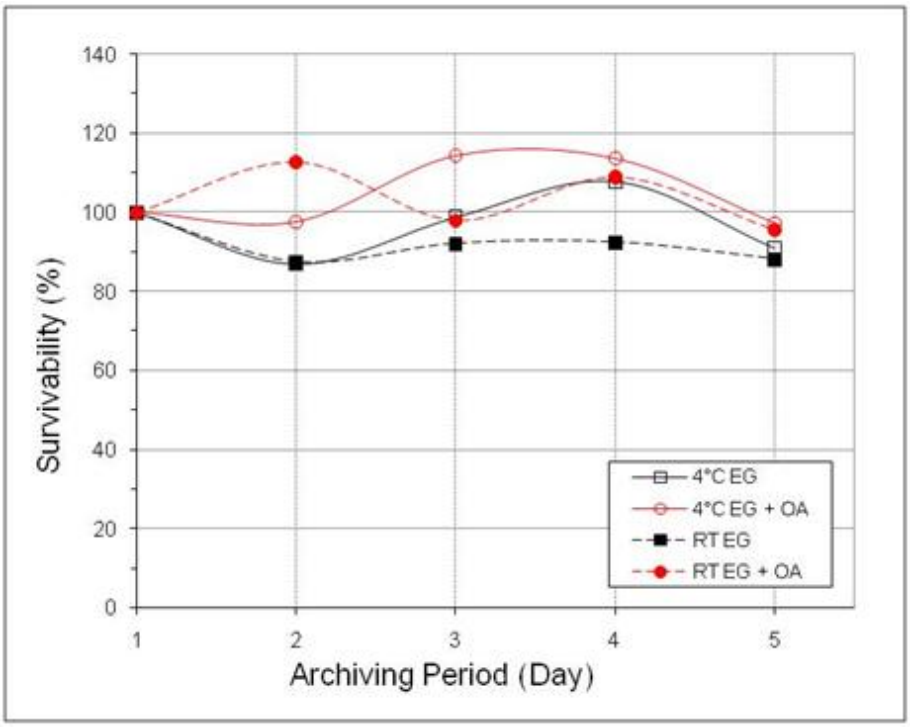

Figure B.17a. Survivability of 100 LPM WWC-collected *E .coli* samples during a five day period in 30% EG, with and without OA at 4^{o}C and RT. See Table A.6 for standard deviation values associated with each data point

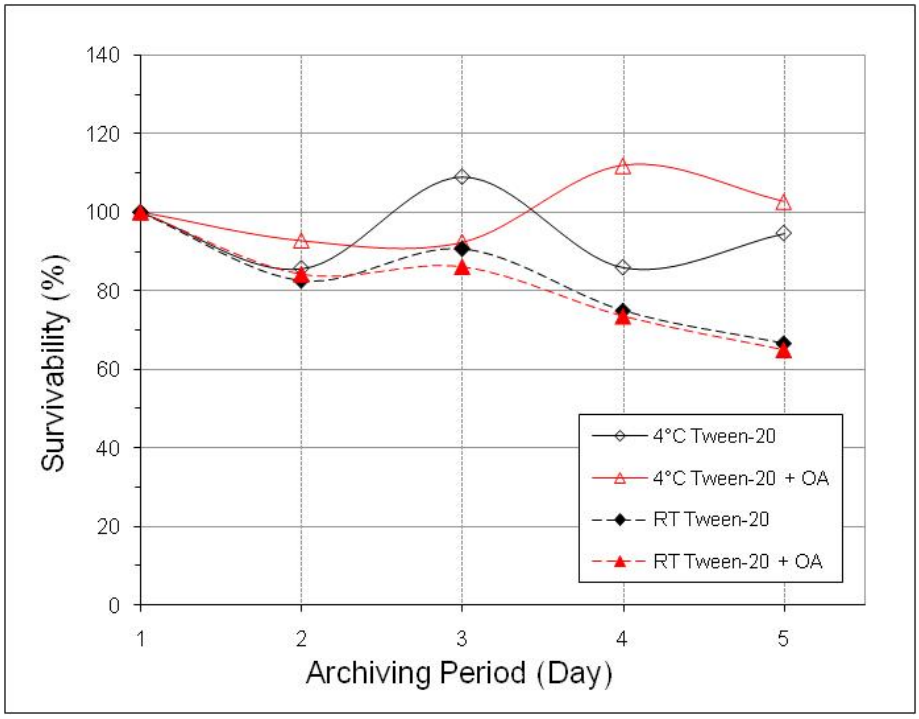

Figure B.17b. Survivability of 100 LPM WWC-collected *E. coli* samples during a five day period in 0.01% Tween-20, with and without OA at 4^{o}C and RT. See Table A.6 for standard deviation values associated with each data point

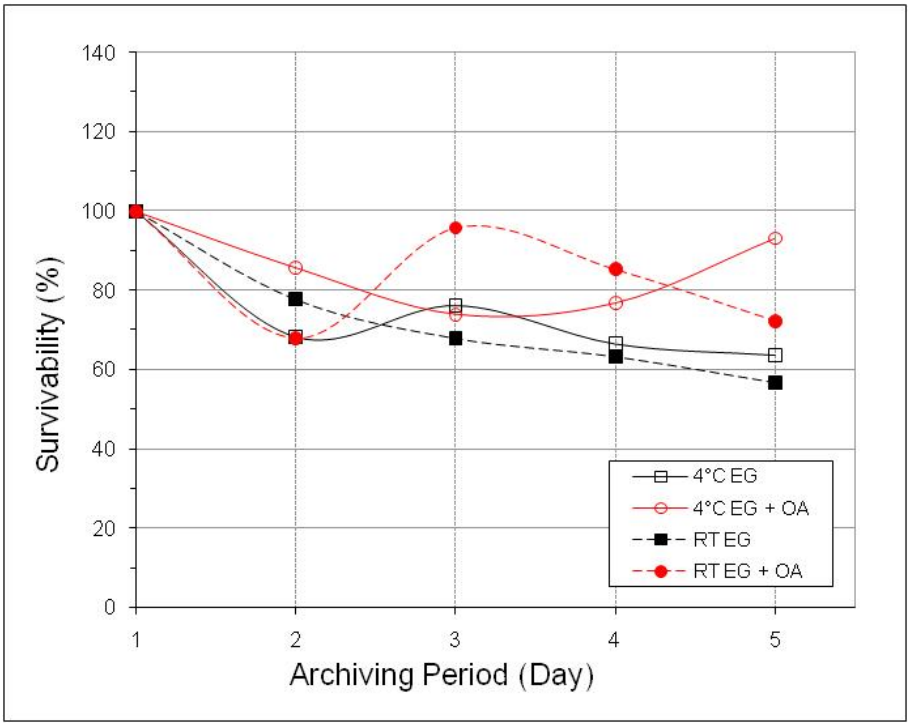

Figure B.18a. Survivability of SKC Impinger-collected *E. coli* samples during five day archiving in 30% EG, with and without OA at 4^{o}C and RT. See Table A.7 for standard deviation values associated with each data point

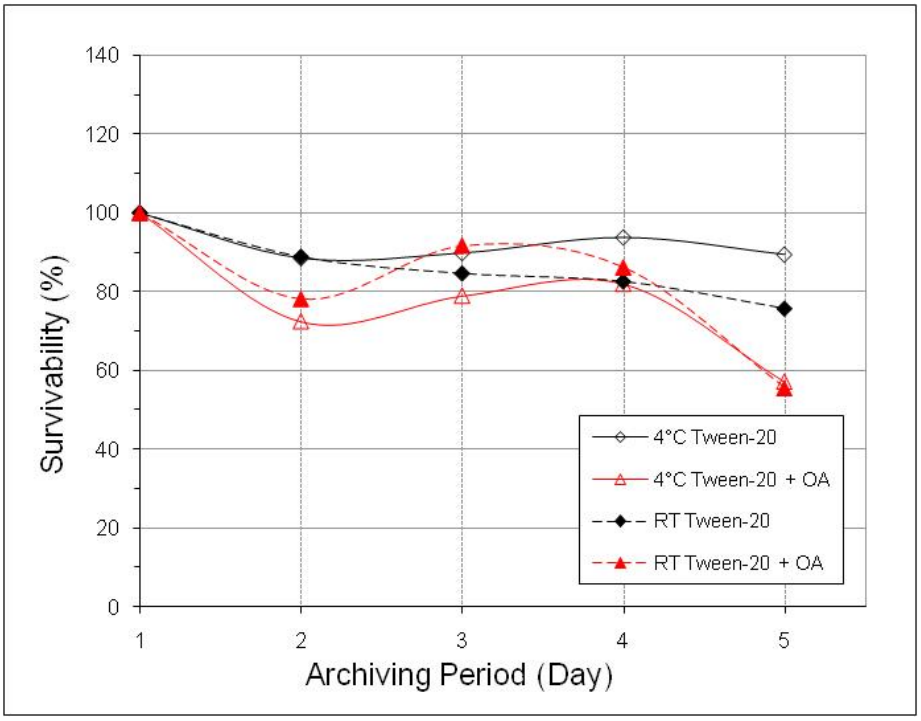

Figure B.18b. Survivability of SKC Impinger-collected *E. coli* samples during five day archiving in 0.01% Tween-20, with and without OA at 4^{o}C and RT. See Table A.7 for standard deviation values associated with each data point

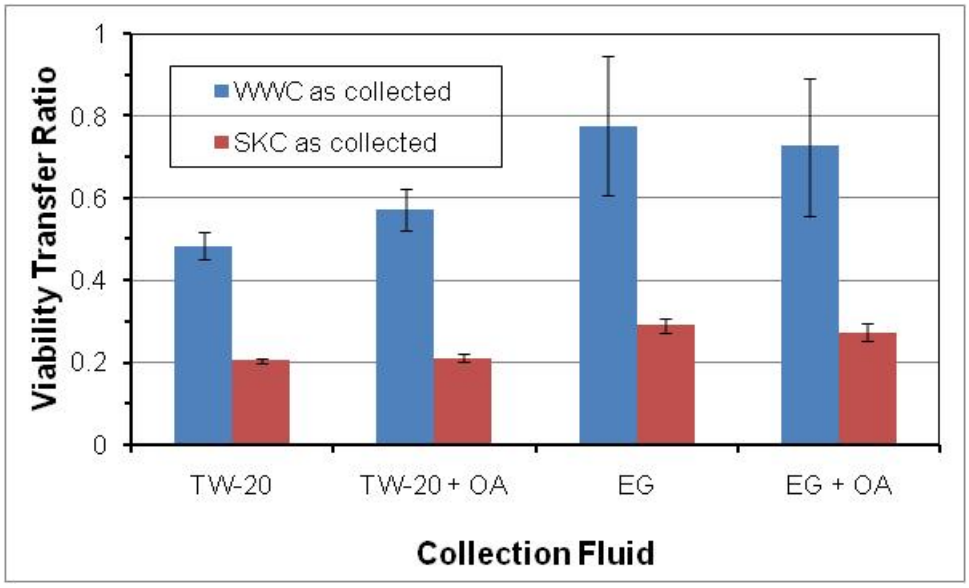

Figure B.19a. Comparison of viability transfer ratio as collected (day one) of *E. coli* samples collected with the WWC and the SKC with the standard deviation shown in the same figure. See Table A.8 for the uncertainty values associated with each data point

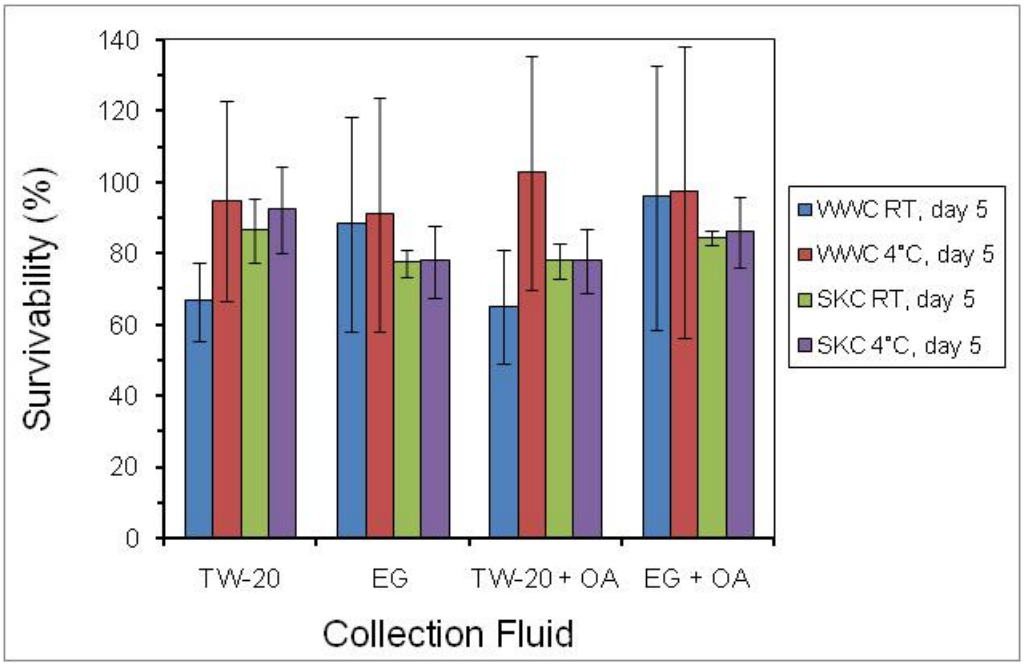

Figure B.19b. Survivability comparison on day five of *E. coli* samples collected with the WWC and the SKC

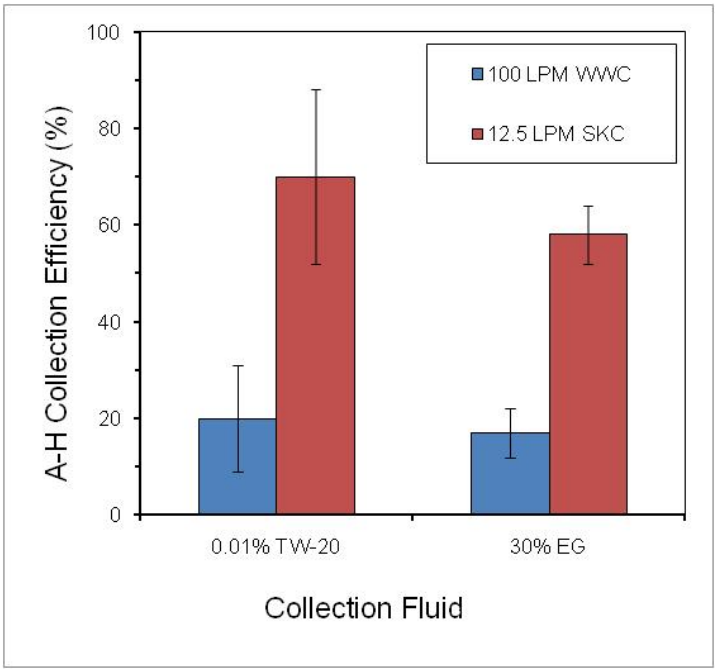

Figure B.20 Aerosol-to-hydrosol collection efficiency for 1µm AD particle size

APPENDIX C

ANALYSIS OF VARIANCE CALCULATIONS

The Analysis of Variance (or ANOVA) can be applied using the following equations:

Among columns, the sum of squares along columns, SS_c is defined as:

$$SS_c = \frac{\sum T_c^2}{nrg} - \frac{T^2}{N} \quad \text{(C.1)}$$

Among rows, the sum of squares along rows, SS_r is defined as:

$$SS_r = \frac{\sum T_r^2}{nrg} - \frac{T^2}{N} \quad \text{(C.2)}$$

Among groups, the sum of squares along groups, SS_g is defined as:

$$SS_g = \frac{\sum T_g^2}{nrg} - \frac{T^2}{N} \quad \text{(C.3)}$$

Column-row interaction implies that the sum of squares, SS_{cr} is defined as:

$$SS_{cr} = \frac{\sum T_{cr}^2}{ng} - \frac{T^2}{N} - SS_c - SS_r \quad \text{(C.4)}$$

Column-group interaction implies that the sum of squares, SS_{cg} is defined as:

$$SS_{cg} = \frac{\sum T_{cg}^2}{nr} - \frac{T^2}{N} - SS_c - SS_g \quad \text{(C.5)}$$

Row-group interaction implies that the sum of squares, SS_{rg} is defined as:

$$SS_{rg} = \frac{\sum T_{rg}^2}{nc} - \frac{T^2}{N} - SS_r - SS_g \quad \text{(C.6)}$$

Column-row-group interaction implies that the sum of squares, SS_{crg} is defined as:

$$SS_{crg} = \frac{\sum T_{crg}}{n} - \frac{T^2}{N} - SS_c - SS_r - SS_g - SS_{cr} - SS_{cg} - SS_{rg} \quad \text{(C.7)}$$

Total sum of squares, SS_{total} is defined as:

$$SS_{total} = \sum x^2 - \frac{T^2}{N} \quad \text{(C.8)}$$

The residual or error, $SS_{residual}$ is defined as:

$$SS_{residual} = SS_{total} - SS_c - SS_r - SS_g - SS_{cr} - SS_{cg} - SS_{rg} - SS_{crg} \quad \text{(C.9)}$$

The mean square, MS is defined as:

$$MS = \frac{SS}{DF} \quad \text{(C.10)}$$

The mean square ratio, MSR is defined as:

$$MSR = \frac{MS}{MS_{residual}} \quad \text{(C.11)}$$

Where,

r = number of rows = number of collection fluid utilized = 4

g = number of groups = number of sample temperature = 2

c = number of columns = number of archiving days = 5

n = number of replications = 3

N = number of observations or sample size

x = sample response = CFU value

T = summation of all sample responses

T_r = summation of sample responses by row

T_c = summation of sample responses by column

T_g = summation of sample responses by group

T_{rg} = summation of sample responses by row and column

T_{cg} = summation of sample responses by column and group

T_{cr} = summation of sample responses by column and row

T_{crg} = summation of sample responses by column, row and group

SS = sum of squares

DF = degree of freedom

MS = mean square

MSR = mean square ratio

Higher experimental mean square ratio (MSR) than the F ratio found in F distribution tables for a certain confidence level i.e. 95 percent confidence indicates a significant difference between the parameters used in experiment.

VITA

Name: Asmaa Sadek Kassab

Address: Aerosol Technology Lab, Texas A&M University,
Mechanical Engineering Dept, TAMU 3123,
College Station, TX 77843-3123

Email Address: askassab@tamu.edu
asmaa_kassab@yahoo.com

Education: B.Sc., Mechanical Engineering, Alexandria University, Egypt, 2006
M.S., Mechanical Engineering, Texas A&M University, 2009